"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL PYRIDINE DERIVATIVES AS ANTI-TUBERCULAR AGENTS AGAINST INHA.,"

A dissertation submitted to

THE TAMIL NADU Dr. M. G. R MEDICAL UNIVERSITY

CHENNAI -600032.

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

BRANCH – II PHARMACEUTICAL CHEMISTRY

Submitted by S.SURESH KUMAR
Reg No: 261615708

Under the guidance of
Dr.A.JERAD SURESH M.Pharm., Ph.D., MBA.,
Principal,Professor and Head,
Department of Pharmaceutical Chemistry

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CERTIFICATE

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EXAMINERS

Acknowledgement

“Gratitude makes sense of our past, brings peace for today and creates a vision for tomorrow”

Foremost, I want to offer this endeavor to our GOD ALMIGHTY for the wisdom he bestowed upon me, the strength, peace of my mind and good health in order to finish this research.

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I would like to express my special gratitude and thanks to my guide **Dr. A.JERAD SURESH, M.PHARM., Ph.D., MBA.,** Principal, Professor and Head, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, for imparting his knowledge and expertise in this study.

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I would like to thank **Dr. S.Rajaraman M.V.Sc., Ph.D**, Special Veterinary officer, MMC and IAEC members for his forceful work for my Acute toxicity study.

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CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

TB	Tubercle Bacillus
WHO	World Health Organization
HIV	Human Immuno Deficiency Syndrome
MDR-TB	Multi Drug Resistant TB
XRD-TB	Extensively Drug Resistant-TB
TDR- TB	Totally Drug Resistant – TB
AIDS	Acquired Immuno Deficiency Syndrome
FDA	Food and Drug Administration
DOTS	Directly Observed Treatment Short-Course
BCG	Bacilli Calmette Guerin
μm	Micro meter
μg/ml	Micro gram/ milli litre
MTB	*Mycobacterium tuberculosis*
DNA	Deoxyribo Nucleic Acid
InhA	Enoyl acyl carrier protein reductase
ATP	Adenosine Tri Phosphate
ADP	Adenosine Di Phosphate
ADME	Absorption, Distribution, Metabolism, Excretion
CADD	Computer Aided Drug Design
QSAR	Quantitative Structural Activity Relationship
QSPR	Quantitative Structure–Property Relationship
HTS	High Throughput screening
SAR	Structural Activity Relationship
MABA	Microplate Alamar Blue Assay
OSIRIS	Optical, Spectroscopic and Infrared Remote Imaging System

PSA	Polar Surface Area
LBDD	Ligand Based Drug Design
SBDD	Structure Based Drug Design
TLC	Thin Layer Chromatography
LC-MS	Liquid Chromatography Coupled with Mass Spectrometry
NMR	Nuclear Magnetic Resonance Spectroscopy
PDB	Protein Data Bank
ADT	AutoDock Tools
Kcal	Kilo Calories
µl	Micro litre
HPLC	High Performance Liquid Chromatography
MIC	Minimal Inhibitory Concentration
OECD	Organization for Economic Co-operation and Development
R_f	Retardation Factor
BACTEC	Bactenecin
Log P	Partition Co-efficient
DMEM	Dulbecco's Modified Eagle Medium

INTRODUCTION

TUBERCULOSIS:

Tuberculosis (TB), is the communicable disease that caused by the bacterium *Mycobacterium tuberculosis*, remains a major public health problem globally. In 2014, more than 9.6 million people are estimated to have fallen ill with TB while 1.5 million people died of the disease.

The disease is closely associated with poverty, which explains the high rates of TB in geographic areas within countries where poverty rates are high. The disease is closely associated with with HIV which has been the major factor for the high rates of TB in many countries [1-2]

LONG TERM COMPLICATIONS OF TB :

Pulmonary TB is associated with various long term lung complications including lung scarring (fibrosis), bronchiectasis, Chronic Pulmonary Aspergillosis (CPA), air way stenosis and Chronic Obstructive Pulmonary Disease (COPD) and it may even be a risk factor for lung cancer.[3-4]

THE ETIOLOGICAL AGENT:

The ***Mycobacterium tuberculosis*** complex includes strains of five species—***M. tuberculosis, M. canettii, M. africanum, M.microti,*** and ***M. bovis*** and two subspecies—***M. caprae*** **and*M. pinnipedii*** . The most notable member of the complex is ***M. tuberculosis*** the causative agent of human tuberculosis which has an exclusive tropism for this host.[5-8]

Fig No:1 Mycobacterium tuberculosis

EPIDEMIOLOGY:

Epidemiology is the study of distribution of disease in society and the factors affecting this distribution.

The epidemiology of tuberculosis varies substantially around the world. The highest rates (100/100,000 or higher) are observed in sub-Saharan Africa, India, China, and the islands of Southeast Asia and Micronesia Estimates provided by USAID in 2007 for South Sudan were 228 cases per 100,000 population. In South Sudan, an estimated 18,500 people develop TB, and 5,300 die of TB annually [9].

Poverty, HIV and drug resistance are major contributors to the resurging global TB epidemic. Approximately 95% of TB cases occur in developing countries. Approximately 1 in 14 new TB cases occur in individuals who are infected with HIV; 85 percent of these cases occur in Africa. An estimated half million cases of multidrug resistant (MDR)-TB also occur annually in Africans; even higher rates of drug resistant disease occur in Eastern Europe[10]

BURDEN OF TB IN INDIA:

- According to WHO 2009 reports, estimated prevalence of TB in India is 3.3 million cases.
- India is the highest TB burden country, an annual 1.98 million incident cases in India.

- In India annual death due to TB is 2,760000. In India death rate due to TB is 1 death per minute.
- In India 2.31 million population living with HIV and 0.9 million population co infected .

CURRENT TREATMENT AGAINST TUBERCULOSIS:

The course of TB treatment based on the stage of infection. TB is usually treated using multiple drugs together in a mixture, with an intensive 2-month initial phase followed by a 4 to 6 -month continuation phase.[11]

Isoniazid (INH), Rifampin (RIF), Pyrazinamide (PZA), and either Ethambutol (EMB) or Streptomycin (SM) are usually the drugs of choice for the treatment of TB.[23,24]. A drug regimen chart created by the Centers for Disease Control and Prevention (CDC) outlines the intervals and doses for drug treatment during the specific phases. [12]

For example, if the MTB isolate is fully susceptible, either EMB or (Streptomycin) SM are discontinued, and PZA can be discontinued after two months of treatment. INH and RIF are continued for four months. Treatment can last from six to nine months, or even up to twenty months. [13]

. TB remains a prominent global health issue and the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) (MDR with added resistance to a fluoroquinolone and an injectable second line agent) strains, and the lack of new effective drugs is of major concern.[14] M. Tuberculosis develops drug resistance exclusively through chromosomal mutations, in particular singlenucleotide polymorphisms and a few through gene environment interaction.[15]

MULTI DRUG RESISTANCE TB :

The drug resistant strains can then spread from person to person like drug susceptible bacteria. Strains of *M. tuberculosis* that are resistant against isoniazid and rifampicin, the most effective drugs against tuberculosis, are defined as multi drug resistant(MDR-TB).

In 2006 the WHO released the first data on extensively drug resistant strains (XDR-TB). These strains are resistant to any fluoroquinolones and at least to one of the injectable drugs kanamycin, capreomycin and amikacin, in addition to isoniazid and rifampicin and occur in every part of the world. Patients infected with XDR-TB are virtually untreatable with current drugs[1]

DIAGNOSIS OF TUBERCULOSIS:

1.Microbiological tests:

2. **Immunological tests:**

(a) ALS (Antibodies from lymphocyte secretions) assay- this is an immunological assay to detect active diseases like tuberculosis, cholera, typhoid etc. [6]

(b) **Mantoux test (MT)/Tuberculin Skin Test (TST)-** positive test indicates infection by TB bacilli, doesn't exclude active disease cases from latent cases and inconclusive in BCG vaccinated people.

3. **Adenosin Deaminase Assay (ADA) test**:

ADA is an enzyme which contributes in purin metabolism and converts adenosine to inosine. ADA is essential for proliferation and differentiation of lymphoid cells, especially T cells, and helps in the maturation of monocytes to macrophages. It seems ADA is an index for cellular immunity. Activity of this enzyme increases in TB, empyema, lymphoma and other chronic inflammatory conditions like Rheumatoid Arthritis (RA). [17]

Need for new anti-TB drugs:

- To improve the treatment of MDR-TB.
- To improve current treatment by shortening the total duration of the treatment.
- The recent rise in TB cases and especially the increase of drug resistant mycobacteria indicate an urgent need to develop new anti-TB drugs.
- There is a need to design new drugs that are more active against slowly growing and nongrowing persistent bacilli.

- Discovery of compound that would reduce both the length of treatment and the frequency of drug administration.
- To provide more effective treatment for latent tuberculosis infection.

New drug to improve current that would reduce both the total length of treatment and the frequency of drug administration.

ENZYME PROFILE:

Among the most attractive molecular targets to the design of novel antibacterial agents are the Fatty Acid Synthase (FAS) pathway enzymes. The Mycobacterium tuberculosis InhA (MtInhA) or 2-trans-enoyl-ACP (CoA) reductase , the fourth enzyme of the type II fatty acid synthase system (FAS II), is one of the key enzymes involved in the elongation cycle of fatty acids in M. tuberculosis.

Its biological role includes the preferentially reduction of long chain enoyl thioester substrates (e.g., containing 16 or more carbon atoms) yielding the long carbon chain of the meromycolate branch of mycolic acids (C40−60), α-branched fatty acids, the hallmark of mycobacteria.8 Previously, it has been shown that InhA is essential to the mycolic acid biosynthesis in Mycobacterium.[18]

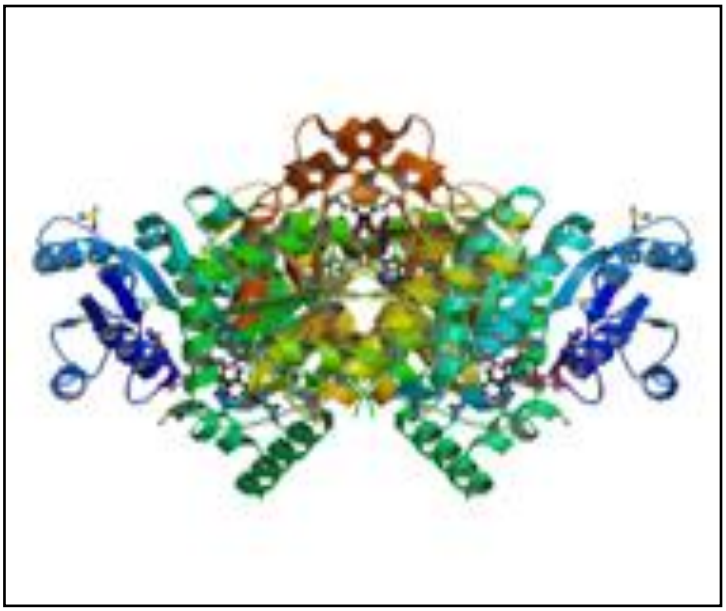


Fig No 2: InhA(Enoyl Acyl Carrier Protein Reductase)

ENZYME NAME	**: ENOYL-ACP REDUCTASE**
CLASSIFICATION	**: OXIDO REDUCTASE**
POLYMER	**: 1**
TYPE	**: Protein**
CHAINS	**: A, B**
ORGANISM	**: Mycobacterium tuberculosis**
PROTEOME	**: Chromosome**
FUNCTIONAL CATEGORY	**: Type II fatty acid biosynthesis pathway**

InhA, the enoyl-ACP reductase in Mycobacterium tuberculosis is an attractive target for the development of novel drugs against tuberculosis, a disease that kills more than two million people each year.

InhA is the target enzyme of the current first line drug isoniazid for the treatment of tuberculosis infections. [18]

Crystal structure of the ternary complex between InhA, NAD(+), and PT70 reveals the molecular details of enzyme-inhibitor recognition and supports the hypothesis that slow onset inhibition is coupled to ordering of an active site loop, which leads to the closure of the substrate-binding pocket.

InhA(enoyl-[acyl-carrier-protein]reductase),involved in mycolic acid synthesis is a target of front-line anti-tubercular drugs, such as isoniazid and ethionamide.[19]

BASIC NUCLEUS INFORMATION:

Pyridine is a basic heterocyclic organic compound with the chemical formula C_5H_5N. In many aspects it can be related to well established and very fundamental aromatic molecule, benzene, with one C-H group replaced by a nitrogen atom. Pyridine has a conjugated system of six π-electrons exactly as benzene has, that are delocalized over the heterocyclic ring.The molecule is planar in nature and follows Huckel criteria for aromaticity.

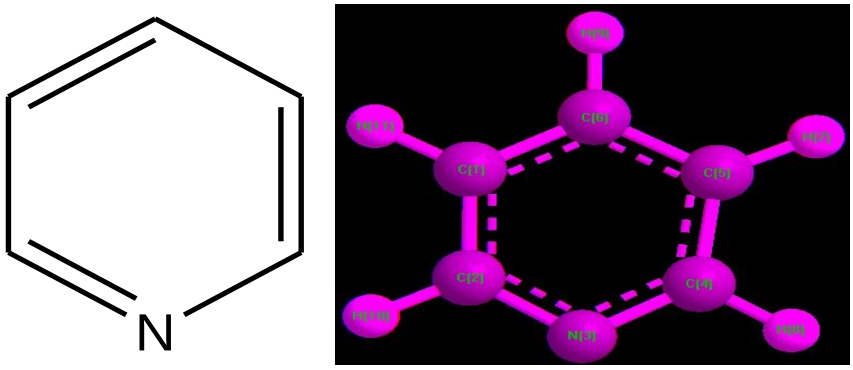


Fig No 3: Structure of Pyridine Nucleus

Physically C_5H_5N Organic base; flammable, toxic yellowish liquid, with penetrating aroma and burning taste; soluble in water, alcohol, ether, benzene, and fatty oils; boils at 116 °C[20]

Pyridines find omnipresent applications in medicaments and in agrochemicals. The leading group are antimicrobials (isoniazid), and histamine h1 antagonists such as pheniramine , but also anticancer, analgesic, and antidepressant agents.

Pyridine derivatives continue to attract great interest due to the wide variety of interesting biological activities observed for these compounds, such as anticancer, analgesic, antimicrobial,

and antidepressant, activities. Pyridine is used in the pharmaceutical industry as a raw material for various drugs, vitamins, and fungicides and as a solvent

Pyridine derivatives have following pharmacological activities:

1.Anticancer activity[21]

2.Antiviral activity[22]

3.Antimicrobial activity.[23]

4.Analgesic activity[24]

5.Antichagastic activity[25]

6.Antifungal activity[26]

7.Antidiabetic activity[27]

8.Antibacterial activity[28]

DRUG DISCOVERY:

The process of drug discovery is very complex and requires interdisciplinary effort to design effective and commercially feasible drugs. Earlier drug discovery has been a trial and error process. The process of drug development has evolved with time. New understanding of the quantitative relationship between structure and biological activity ushered the beginning of computer –aided drug design with the help of computers, a new era has begun in drug discovery. The development cost will be cut by almost third. The development times are reduced. [29]

LEAD AND LEAD OPTIMIZATION:

A lead is defined as a compound, usually a small organic molecule that demonstrates desired biological activity on a validated molecular target. Lead optimization is technique of refining 3D structures of drug molecules and promoting the binding of drug to protein active sites.

In this technique modification of a structure of the drug molecules is done by docking every specific structure of a drug compound in active site of protein and calculating the extent of the interaction.[30] Optimization aids in the several modification of newer molecules in order to improve the physico-chemical properties and biological activity for a given set of compounds in

the library. Further structural modification improves the affinity, reactivity towards target and enhances stability during metabolism.[29]

TYPES OF DRUG DESIGN:

Advances in computation techniques and hardware have facilitated the application of in-silico methods in the discovery process drug design can be categorized as two types

- Structure based drug design
- Ligand based drug design

Structure based drug design:

SBDD is the approach where the structural information of the drug target is exploited for the development of inhibitors receptor structure(s) is a prerequisite for this method.Most commonly the structure of the receptor is determined by experimental techniques such as X-ray crystallography or NMR. If the structure of the protein drug target is not available, protein structure can be predicted by computational methods threading and homology modelling.

Ligand based drug design:

It is also called indirect drug design. Ligand based drug design is an approach used in the absence of the receptor 3D information and it relies on knowledge of molecules to the biological target of interest. 3D quantitative structure activity relationship (3D QSAR) and pharmacophore modelling are the most important and widely used tools in the ligand based drug design. They can provide protective models suitable for lead identification and optimization.[31]

COMPUTER AIDED DRUG DESIGN

Computer aided drug design use as computational chemistry to discover, enhance or study drugs and related biologically active molecules. Molecular mechanics or molecular dynamics are most often used to predict the confirmation of the small molecule and to model conformational changes in the biological target but may occur when the small molecules binds to it.

Molecular mechanics methods may also be used to provide semi quantitative prediction of the binding affinity also knowledge based scoring function may be used to provide binding affinity estimates.[32]

Drug design with the help of computers may be used at any of the following stages of drug discovery

- **Hit identification** using virtual screening (structure or ligand based-based design)
- **Hit-to-lead optimization** of affinity and selectivity (structure based design, QSAR, etc)
- **Lead optimization**, optimization of other pharmaceutical properties while maintaining affinity.

In order to overcome the insufficient prediction of binding affinity calculated by resent scoring functions, the protein-ligand interaction and compound 3D structure are used to analysis[33]

AIM AND OBJECTIVE

AIM:

To develop the novel and potent anti-tubercular agents against InhA enzyme (Enoyl acyl carrier protein reductase)

OBJECTIVE:

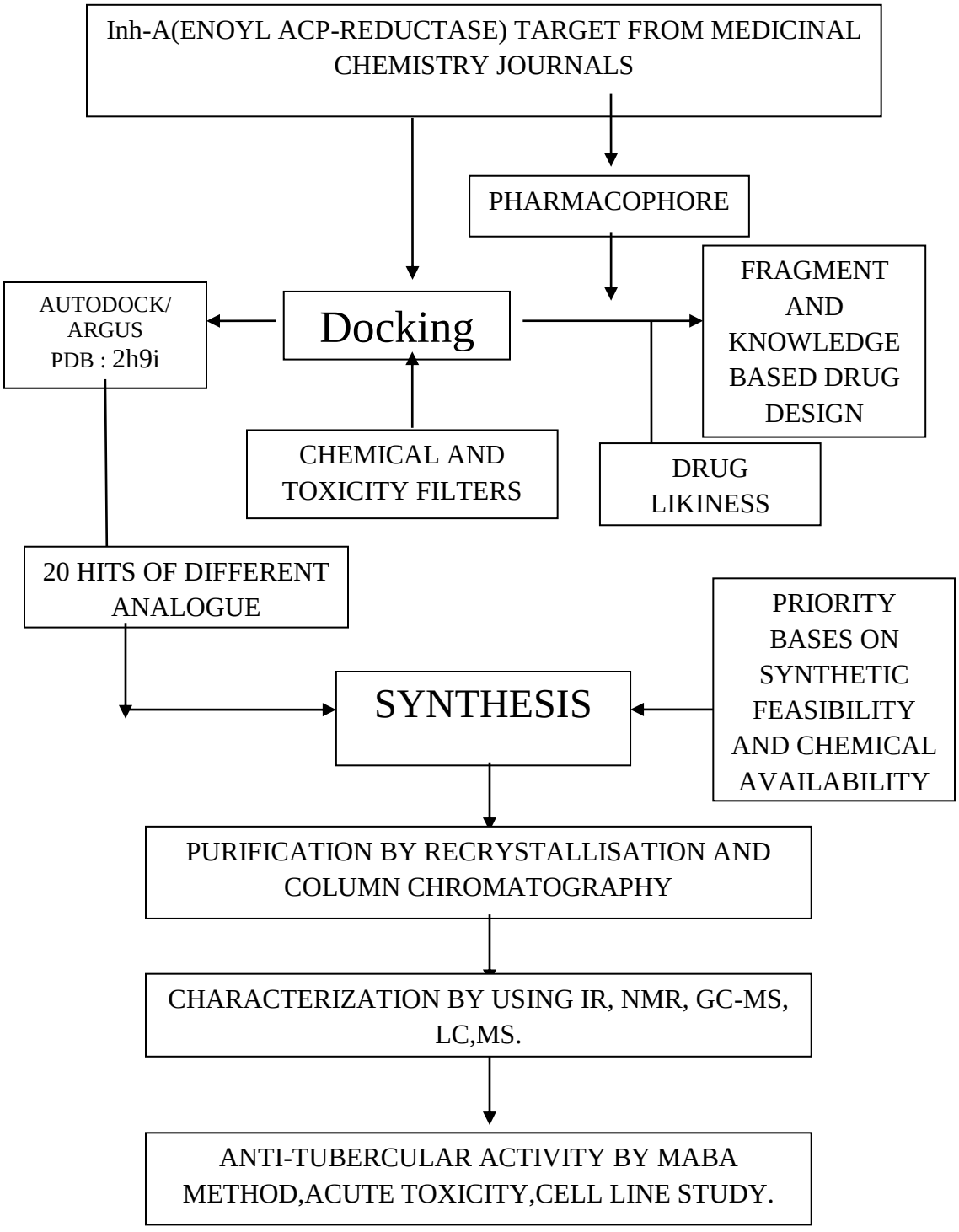
To design molecules, synthesize, characterize and determine antitubercular activities, cytotoxicity and acute toxicity.

The plan of work includes:

- Design of InhA (enoyl-Acyl Carrier Protein reductase) inhibitors by docking studie susing Autodock (1.5.6 version) software.
- Insilico Drug likeness prediction.
- Insilico Toxicity Assessment.
- Laboratory synthesis of those compounds with top Docking Scores.
- Characterization of the synthesized compounds by
    - Infrared Spectroscopy.
    - H1 Nuclear Magnetic Resonance Spectroscopy.
    - Melting point.
    - GC-Mass Spectroscopy.
    - LC-Mass Spectroscopy.
- In-vitro anti -tubercular activity of synthesized compounds (MABA).
- Acute Toxicity on mice
- Cytotoxicity on animal kidney cell.

PLAN OF WORK

The present study will be perform on the basis of flow chart given below:



LITERATURE REVIEW

In order to know the current status regarding the advances in TB the literature pertaining to the disease, design, synthesis, characterisation and biological evaluation were reviewed.

LITERATURE REVIEW FOR TARGET ENZYME:

1.Banerjee et al., 1994 reported the wild-type inhA gene of M. tuberculosis or M. smegmatis was shown to confer INH resistance and ethionamide (ETH) resistance to M. smegmatis and to Mycobacterium bovis BCG when transferred on a multicopy .Moreover, a point mutation (causing the amino acid substitution S94A) within the inhA genes of an INH-resistant M. smegmatis and an INH-resistant M. bovis mutant was shown to be sufficient to transfer INH and ETH resistance to M. smegmatis when transferred by allelic exchange within M. smegmatis.[34]

2.Heym et al., 1994., Ristow et al., 1995., reported the numerous groups have identified mutations from INH-resistant clinical isolates of M. tuberculosis within the promoter of inhA and the inhA protein product that are consistent with the premise that inhA encodes the target of INH and ETH in M. tuberculosis.[35]

3.Johnsson and Schultz, 1994 the inhA gene was predicted to encode an enoyl-ACP reductase of the fatty acid synthase II (FASII) system of mycobacteria. In an in vitro mycolic acid synthesis assay, KatGactivated INH inhibited the activity of purified InhA protein.[36]

4.Mdluli et al. (1998)A biochemical approach was used to identify another gene involved in INH resistance, kasA, which encodes a b-ketoacyl-ACP synthase This protein was reported to be covalently associated with INH and ACP. Using radioactive INH, the activated INH binds ACP in M. tuberculosis cells, but it remains unclear what the chemical nature of this binding is and whether it is relevant to INH action.[37]

REVIEW OF LITERATURE RELATED TO THE EVALUATION OF ANTI TUBERCULAR ACTIVITY BY MABA :

5. Scott G Franzblau. et al. studied MIC determination by MABA. A colorimetric, Microplate Based Alamar Blue Assay (MABA) method was used to determine the MICs of Isoniazid, Rifampin, Streptomycin and Etambutol for 34 peruvian Mycobacterium tuberculosis isolates and the H37Rv strain by using bacterial suspensions prepared directly from media. The MABA is a simpe, rapid, low cost, appropriate technology which does not require extensive instrumentation and which makes use of a nontoxic, temperature stable reagent [38].

6. Sephra N Rampresad. et al. studied the various applications of Alamar Blue as an indicator. Alamar Blue is a redox indicator that is used to evaluate metabolic function and cellular health. The Alamar Blue Bioassay is being utilized to access cell viability and cytotoxicity in a range of biological and environmental system and in a number of cell types including bacteria, yeast, fungi, and protozoa.[39]

7. Jose de Jesus Alba-Romero et al. applied the Alamar Blue Assay to determine the susceptibility to anti-tuberculosis pharmaceuticals.[40]

LITERATURE FOR TUBERCULOSIS:

8. Rahul Jain et al. (2005) have shown that Tuberculosis (TB) is one of the most devastating diseases primarily due to several decades of neglect, and presents a global health threat of escalating proportions. TB is the second leading infectious causes of mortality today behind only HIV/AIDS.[41]

9 James C Sacchettini et al. (2004) worked on TB drug discovery. Addressing issues of persistence and resistance by reviewing the recent developments of some of the pathways involved in a persistent infection and pathogenesis of mycobacterium tuberculosis, which reveal new targets for drug development. [42]

LITERATURE REVIEW FOR DRUG DESIGN :

10.Wermuth C G ., (2006) reviewed the similarity in drugs with the importance and reflections on analogue design. He also clarified the terminology of analogue design by establishing a clear distinction among three kinds of analogues.[43]

11Ghorpade S R *et al* ., (2013) studied a pharmacophore-based search which led to the identification of thiazolopyridine urea as a novel scaffold with antitubercular activity acting through inhibition of DNA Gyrase B (GyrB) ATPase. [44]

12. **Kore P P** *et a***l**., (2012) reported a brief history of CADD, DNA as target, receptor theory, structure optimization, structure-based drug design, virtual high-throughput screening (vHTS) and graph machines. [45]

13.**Frederick W G** *et al* , (2015) reported the general principles that should be applied to ensure the building block collection's impact on drug discovery projects.[46]

LITERATURE REVIEW FOR SPECTROSCOPY :

14.Gurdeep R. Chatwal *et al.,*(2005) Text book on Instrumental methods of chemical analysis.47

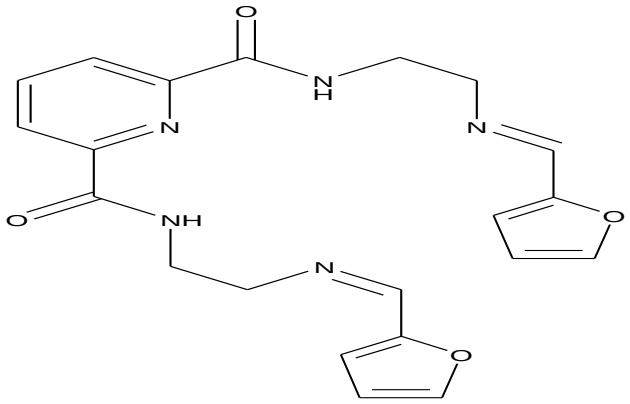
15.P.S.Kalsi*et al.,*(2007)Text book on Spectroscopy of organic compounds.[48]

16.D.Kealey*et al*.,(2010)Text book on Instant notes Analytical Chemistry.[49]

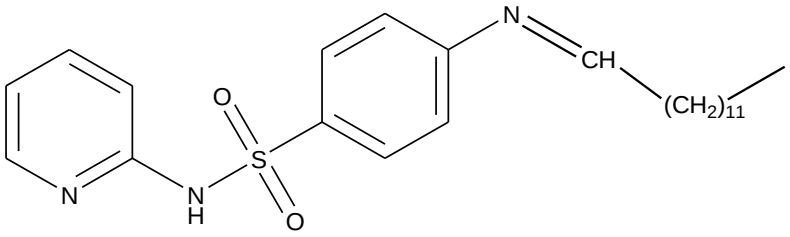
17.Y.R.Sharma,*et al.,*(2008)Text book on Elemental Organic Spectroscopy.[50]

LITERATURE REVIEW FOR PYRIDINE:

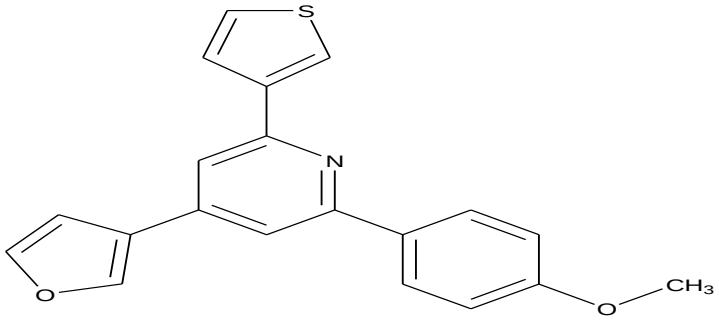
18. **Xia et al., 2006**., reported a series of novel Schiff base derivatives with different substituent were screened for antibacterial activity against S. areus. Synthesized compounds showed a significant antibacterial activity [51]



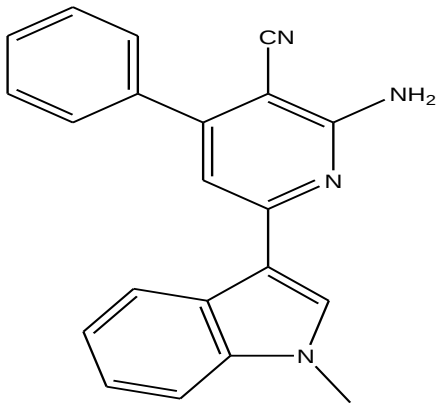
19. **Mohsen et al.,** reported a synthesis of series of sulfapyridine-polyhydroxyalkylidine (or arylidene)-imino derivatives (Schiff's bases) was presented by having considerable cytotoxic effect against breast carcinoma cell lines MCF7 and cervix carcinoma cell line HELA in comparison with5-flurouracil and doxorubicin.[52]



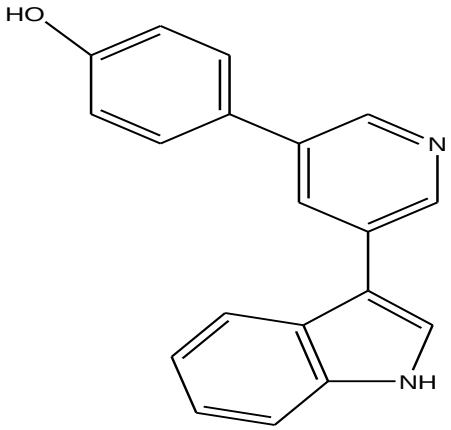
20**.Lee et al** synthesized a series of 2-(thienyl-2-yl or - 3-yl)-4-furyl-6-aryl pyridine derivatives and evaluated for their topoisomerase I and II inhibition and cytotoxic activity against human cancer cell lines. Compounds were showed moderate topoisomerase I and II inhibitory activity and showed significant topoisomerase II inhibitory activity. Most significant cytotoxicity against Hela, K562, HCT15, and MCF-7 cell lines, was shown by the compound [53]



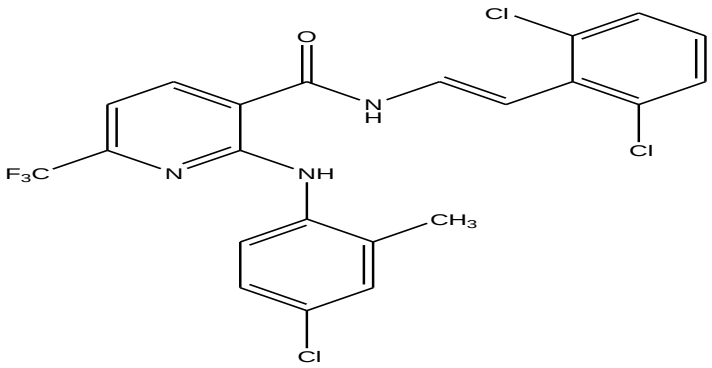
21. **Fan Zhang et al.** (2011), synthesized in vitro anti-tumor activity of 2-amino-3 cyano-6-(1 H -indol-3- yl)-4-phenyl pyridine derivative.[54]



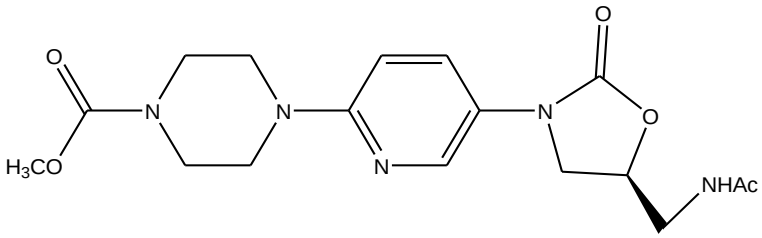
22. **Ulrich Jacquemard et. al.** Synthesized 3,5-bis(2-indolyl)pyridine and 3-[(2-indolyl)-5-phenyl]-pyridine derivatives as CDK inhibitors and cytotoxic agents. [55]



23. **Onnis et al.,** synthesized and evaluated the anticancer activity of 2 arylamino-6-trifluoromethyl-3- (hydrazonocarbonyl) pyridines. The potent compound was 2, 6-dichlorobenzaldehydehydrazone (11), which inhibited the growth of all tested cancer cell lines with nanomolar potency, without having any animal toxicity [56] .

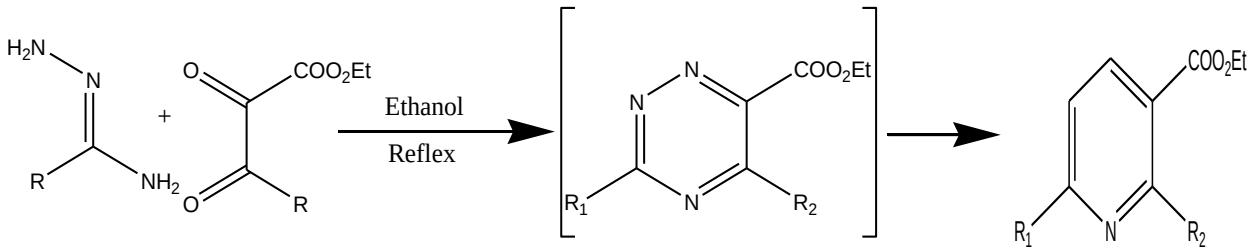


24. **Cui et al.,** synthesized a series of substituted piperazinyl pyridyl oxazolidinone compounds and evaluated them against Gram positive organism (Staphylococci, Streptococci, Enterococci).[57]

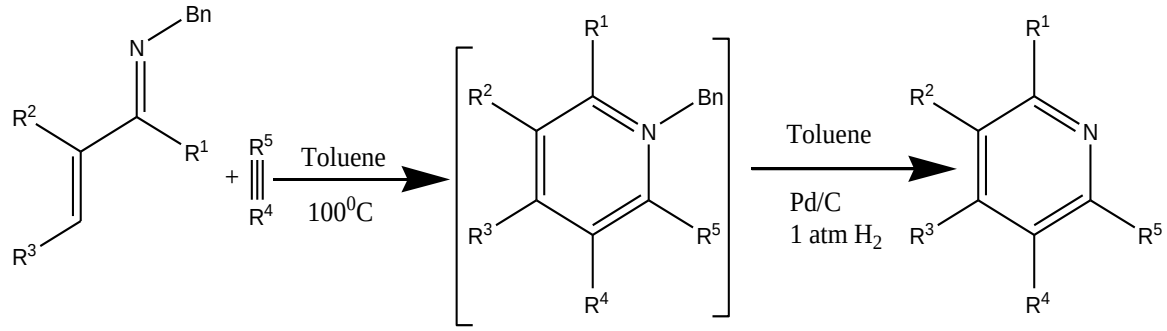


LITERATURE REVIEW FOR DESIRED CHEMICAL ENTITIES:

25.**Stanforth et al.** have developed a single-step synthesis of alkyl, aryl, heteroatom, and ester 2,3,6-trisubstituted pyridines in moderate to good yield from amidrazones, a,b-diketoesters, and 2,5-norbornadiene [58]

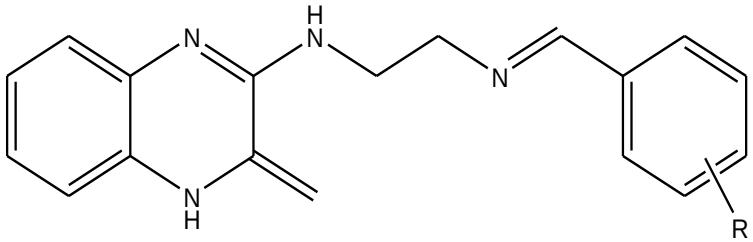


26.**Ellman et al.** have developed a single-step, transitionmetal- catalyzed [4+2] method for the synthesis of di-, tri-, tetra-, and pentasubstituted pyridines from a,b-unsaturated imines and alkynes.[59]



LITERATURE REVIEW FOR SCHIFF BASES:

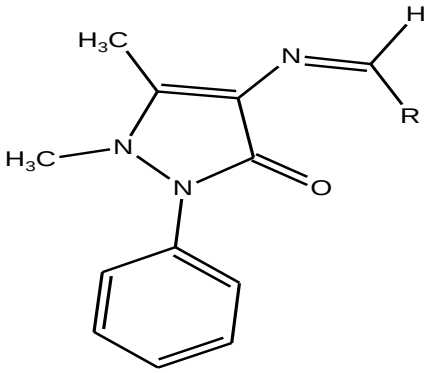
27.**Ghadage et al.** reported some novel Schiff's bases of 3-{[2-({(E)-[substituted) phenyl] methylidene}amino)ethyl]amino}quinoxalin-2(1*H*)-one and evaluated for *in vivo* anticonvulsant activity by using pentylene tetrazole-induced seizure model.[60]



R= Cl, NO2, OH, OCH3, N(CH3)2

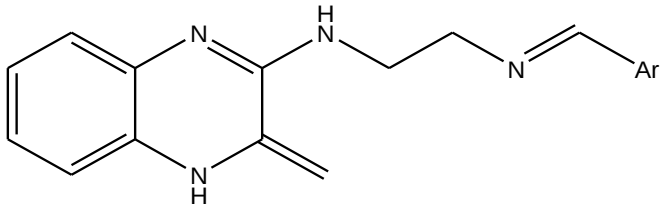
28. **Asiri et al**. synthesized a series of 1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-onecontaining Schiff's bases and screened for their antibacterial activities. 1,5-dimethyl-2-

phenyl-1,2-dihydro-3*H*-pyrazol-3-one Schiff's base derivatives were prepared by the reaction of 4-aminophenazone with different substituted aromatic aldehydes [61].



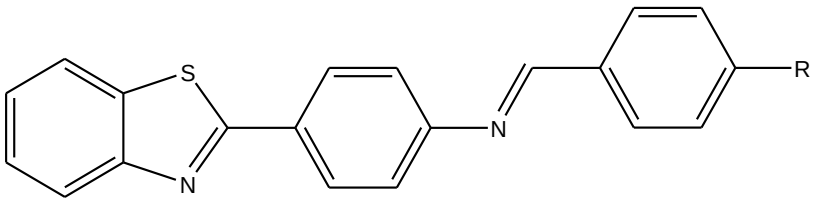
R= 2-Cl-C6H4, 2CN-C6H4, 2-OCH3-C6H4, 4-N(CH3)-C6H4 etc.

29. **Ghadage et al.** synthesized a novel series of 3-[substituted(phenylmethylidene] amino)ethyl)amino] quinoxalin-2(1*H*)-one (from [3-(2-aminoethyl)amino]quinoxalin- 2(1*H*) one and aromatic aldehydes. Compounds were screened for *in vitro* anti-inflammatory activity using carrageenan-induced paw model and produced significant inhibition of paw oedema [62].



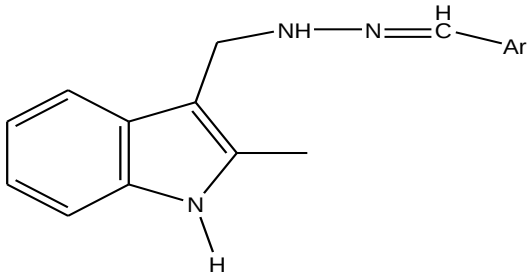
Ar= C6H5, 3-NO2-C6H4, 2-OH-C6H4, 4-OCH3-C6H4, CH=CHCH2C6H5

30. **Hutchinson et al.** synthesized fluorinated analogues of 2-(4-aminophenyl) benzothiazoles among which 2-(4-amino-3-methylphesnyl)-5-fluorobenzothiazole exhibited selective and potent anticancer activity.[63]

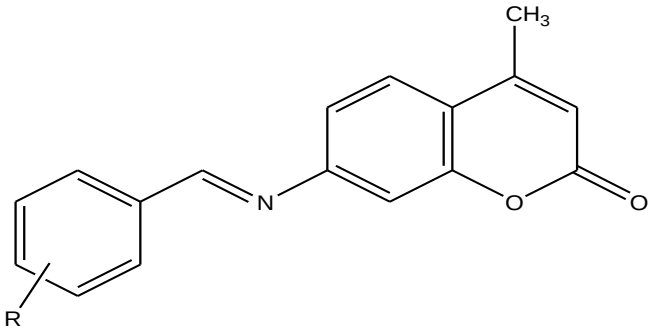


R= Cl, Br, F etc.

31. **Rapolu et al.** reported synthesis of a series of Schiff bases 2-methyl -1-*H* indole-3-carbohydrazide and *N*-benzylidine-2-methyl-1-*H*indole-3-carbohydrazide as anti-inflammatory agents.[64]



32. **Ronad et al.** prepared a series of 7-(2-substituted phenylthiazolidinyl)-benzopyran-2-one de rivatives of Schiff bases , and evaluated for anti-bacterial and anti-fungal activities against various bacterial and fungal Anand et al : Schiff bases: A Review on Biological Insights 865 strains.[65]



From the literature review the following points are concluded

- TB is the deadliest disease across the world wide which has to be treated.
- Inh A (enoyl ACP- Reductase) is the most attractive target enzyme to treat Mycobacterium tuberculosis.

It is clear that ***pyridine*** scaffold has a tremendous activity against the Mycobacterium tuberculosis from the literature review.

MATERIALS AND METHODS

COMPUTER AIDED DRUG DESIGN :

A binding interaction between a small molecule ligand and an enzyme protein results in activation or inhibition of the enzyme, which results in agonism or antagonism. Identification of new ligands for a given receptor by searching largedatabases of 3D structures of small molecules to find those fitting the binding pocket of the receptor using docking programs. This method is known as virtual screening.[66]

DOCKING:

Docking program is used to fit the ligand molecule into the target structure in a variety of position, conformations and orientations. Docking mode is known as pose. Each pose scored based on its complementarities to the target in terms of shape and properties such as electrostatics in order to identify the most favorable energetic pose.

SCORING FUNCTIONS[67]:

One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Böhm. This empirical scoring function took the form:

ΔG0 – empirically derived that in part corresponds to the overall loss of translational and rotational entropy of the ligand upon binding.

ΔGhb – contribution from hydrogen bonding

ΔGionic – contribution from ionic interactions

ΔGlip – contribution from lipophilic interactions where is surface area of lipophilic contact between the ligand and receptor

ΔGrot – entropy penalty due to freezing a rotatable bond in the ligand bond upon binding

$$\Delta G bind = -RT \ ln \ Kd$$

$$Kd = \frac{[\mathbf{Ligand}]\ [\mathbf{Receptor}]}{[\mathbf{Complex}]}$$

$$\Delta G bind = \Delta G \ desolvation + \Delta G \ motion + \Delta G \ configuration + \Delta G \ interaction$$

Where:

ΔG desolvation is the enthalpic penalty for removing the ligand from solvent

ΔG motion is the entropic penalty for reducing the degrees of freedom when a ligand binds to its receptor

ΔG configuration is the conformational strain energy required to put the ligand in its "active" conformation

ΔG interaction is the enthalpic gain for "resolvating" the ligand with its receptor.

According to Gibbs free energy equation, the relation between dissociation equilibrium constant, Kd, and the components of free energy was built.

MOLECULAR DOCKING BY AUTODOCK®:

Autodock® 4.2.5.1 is an automated procedure for predicting the interaction of ligands with biomacromolecular targets. Progress in biomolecular x-ray crystallography continues to provide important protein and nucleic acid structures. These structures could be targets for bioactive agents in the control of animal and plant diseases, or simply key to the understanding of fundamental aspects ofbiology. The precise interaction of such agents or candidate molecules with their targets is important in the drug discovery process.

In any docking scheme, two conflicting requirements must be balanced: the desire for a robust and accurate procedure, and the desire to keep the computational demands at a reasonable level. The ideal procedure would find the global minimum in the interaction energy between the substrate and the target protein and exploring all available degrees of freedom (DOF) for the system.

AutoDock® combines two methods to achieve these goals: rapid grid-based energy evaluation and efficient search of torsional freedom. The current version of AutoDock® using the Lamarckian Genetic Algorithm and empirical free energy scoring function, typically will provide reproducible docking results for ligands with approximately 10 flexible bonds. The quality of any docking results depends on the starting structure of both the protein and the potential ligand. The protein and ligand structure need to be prepared to achieve the best docking results.

- **Protein preparation**
- **Ligand preparation**
- **Receptor grid generation**
- **Ligand docking (screening)**

DOCKING PROCEDURE

Preparation of protein:

- Read molecule from the file (allows reading of PDB coordinate files.)
- Edit -Charges – Compute Gasteiger (for arbitrary molecules)
- Edit – Hydrogen –Merge non polar
- Save as .**pdb** in AutoDock® folder

Preparation of Ligand:

- Docking – docking parameters: opens a panel for setting the parameters used during the docking calculation, including options for the random number generator, options for the force field, step sizes taken when generating new conformations, and output options.
- Ligand –Input from file
- Ligand – Torsion –choose torsion: Rotatable bonds are shown in green, and non-rotatable bonds are shown in red. Bonds that are potentially rotatable but treated as rigid, such as amide bonds and bonds that are made rigid by the user, are shown in magenta.
- Ligand – Torsion –set number of torsion: sets the number of rotatable bonds in the ligand by leaving the specified number of bonds as rotatable.
- Ligand – Output – save as **.pdbqt** in AutoDock folder

Preparation of Docking Parameters:

- Docking –Open the macromolecules – set rigid file name.
- Docking – ligand – open the ligand.
- Docking –search parameters – genetic algorithm parameters : this command opens a panel for setting the parameters used by each of the search algorithms, such as temperature schedules in simulated annealing and mutation/crossover rates in genetic algorithms.
- Docking- output –Lamarkian GA –save as **.dpf (**docking parameterfile**)** Open command prompt **[autodock4.exe –p a.dpf –l a.dlg]**

Visualization / Interpretation of Docking:

- Analysis –Docking – open .dlg (docking log file) file
- Analysis – open the macromolecule
- Analysis – Confirmation –Play and Play ranked by energy : Play- will use the order of conformations as they were found in the docking calculations, and Play Ranked By Energy will order the conformations from lowest energy to highest energy.
- Analysis – Load : Information on the predicted interaction energy is shown at the top and the individual conformations
- Analysis – Docking – show interaction: specialized visualization to highlight interactions between the docked conformation of the ligand and the receptor.

LIPINSKI'S RULE[68]

Variants

In an attempt to improve the predictions of druglikeness, the rules have spawned many extensions,

1. Partition coefficient log P in −0.4 to +5.6 range
2. Molar refractivity from 40 to 130
3. Molecular weight from 180 to 500
4. Number of atoms from 20 to 70 (includes H-bond donors [e.g. OHs and NHs] and H-bond acceptors [e.g. Ns and Os])

Also the 500 molecular weight cutoff has been questioned. Polar surface area and the number of rotatable bonds has been found to better discriminate between compounds that are orally active and those that are not for a large data set of compounds in the rat.

In particular, compounds which meet only the two criteria of:

1. 10 or fewer rotatable bonds and
2. Polar surface area no greater than 140 A^2

are predicted to have good oral bioavailability.

IN-SILICO TOXICITY PREDICTION OSIRIS® :

In silico toxicity prediction is done using **OSIRIS®** Property Explorer. It is a free software available for access in the Organic Chemistry Portal. Using this prediction tool, mutagenicity, tumerogenicity, skin irritation and reproductive effects can be calculated. The prediction properties relies on a precompiled set of structure fragment that gives rises to toxicity alerts in case they are encountered in the structure currently drawn. These fragment lists is created by rigorously shredding all compounds in the data base known to be active in a certain toxicity class. During the shredding any molecule is first cut at every rotatable bonds leading to a set of core fragments.

MOLINSPIRATION® :

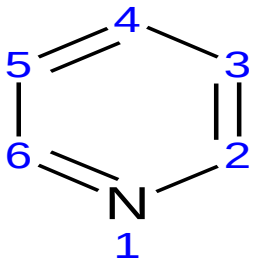
The designed and docked molecules are screened in silico using **MOLINSPIRATION®** Cheminformatics software to evaluate drug likeness. This tool is quick and easy to use. It is a software available online for calculation of important molecular properties log P, polar surface

area, number of hydrogen bond donors and acceptors and others, as well as prediction of bioavailability score for the most important drug targets (GPCR ligands, Kinase inhibitors, ion channel modulators, nuclear receptors).

HETEROCYCLIC CHEMISTRY:

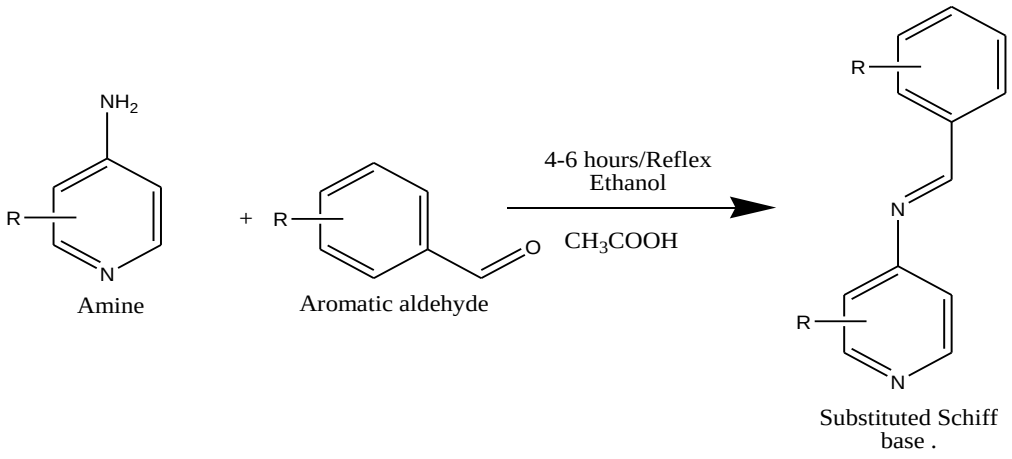
In the area of new drug development heterocyclic derivatives are evaluated for their various biological actions. Heterocyclic derivatives are found to have significant pharmacological activities. Among various compounds I have chosen Pyridine a six membered ring containing nitrogen at position 1.

PYRIDINE NUCLEUS:



SYNTHETIC SCHEME:-

- **Synthesis of Schiff base:**



Procedure:

An equimolar quantity of 3,5-dichloro 4-amino pyridine [0.01mole] was added to various Aldehydes [0.01mole] and dissolved in absolute ethanol, few drops of glacial acetic acid was added as a catalyst, the reaction mixture was reflexed for 4-6 hours on completion of the reaction was monitored by TLC, cooled to room temperature and the reaction mixture was poured into crushed ice, the product was formed, filterd, dried and recrystallised using ethanol.

Amines used:

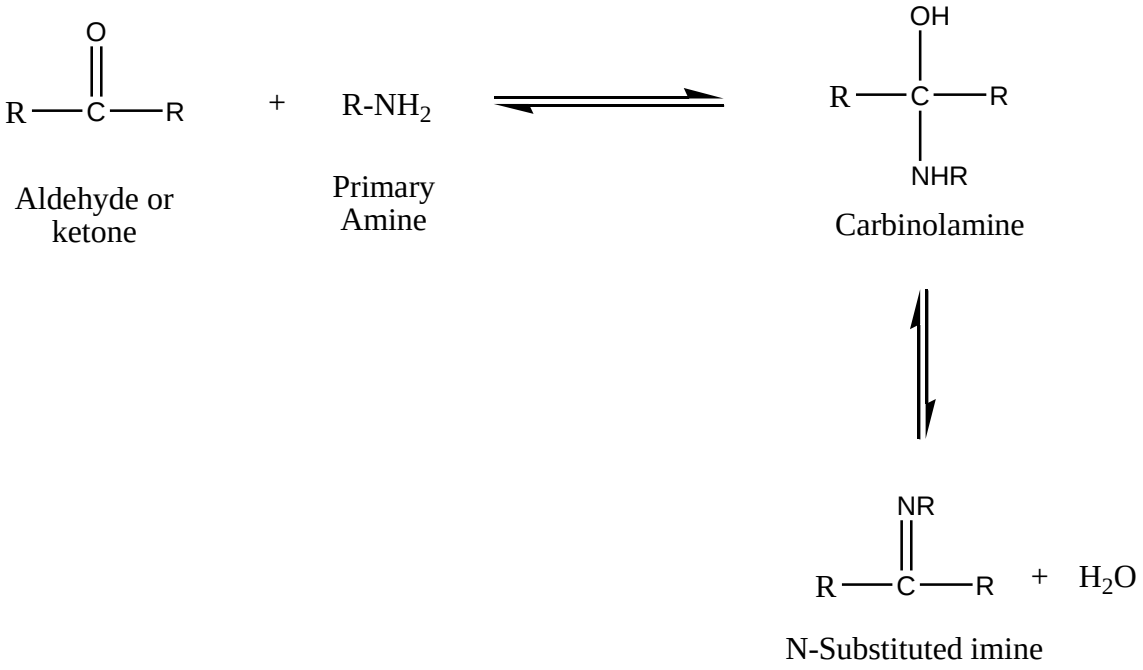
- 3,5-dichloro 4-amino pyridine
- 2-amino 4-methyl pyridine.

Aldehydes used:

- 3-nitro benzaldehyde
- 4-chloro benzaldehyde
- Parahydroxy benzaldehyde
- Indole 3-carbaldehyde

Mechanism :

The formation of a Schiff base from an aldehydes or ketones is a reversible reaction and generally takes place under acid or base catalysis, or upon heating.



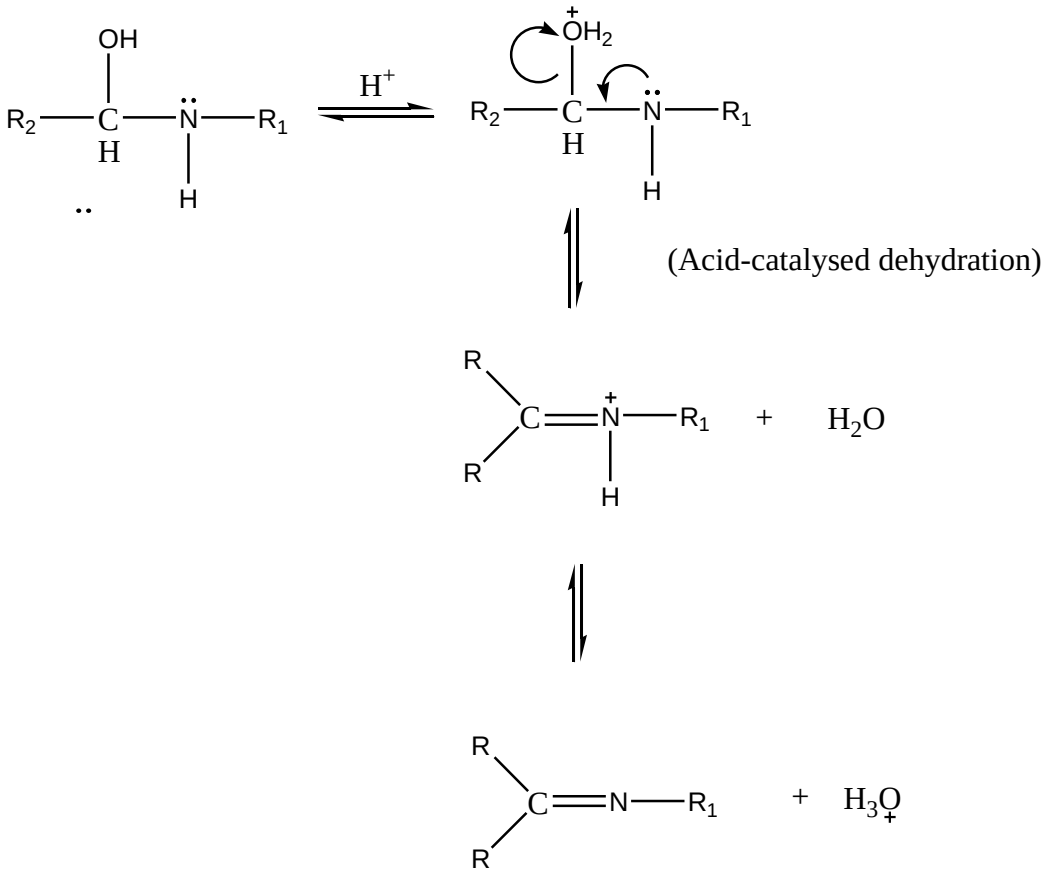
Aldehyde or ketone

Primary Amine

Carbinolamine

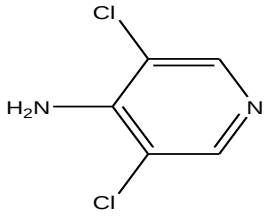
N-Substituted imine

The mechanism of Schiff base formation is based on the neucleophilic addition to the carbonyl group. In this case, the neucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine.The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration.


OH
R2
C
H
N
R1
H
H+
OH2
(Acid-catalysed dehydration)
R
C
N
R1
H2O
H3O+

REACTANT PROFILE

1.3,5-DICHLORO 4-AMINO PYRIDINE:



Synonym: 3,5-dichloro 4-pyridinamine

Molecular Formula: $C_5H_4C_{l2}N_2$

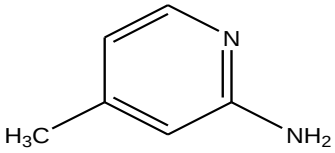
Formula weight: 163

Melting point:159^0C

Boiling point:

Solubility : Chloroform , DMSO, Methanol.

2. 2-AMINO 4-METHYL PYRIDINE:



Synonym : 4-methyl 2-pyridinamine

Molecular Formula **:** $C_6H_8N_2$

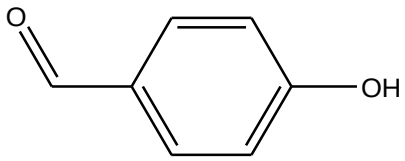
Formula weight **:** 108.14

Melting point **:** 97-101°C

Boiling point **:** 231^0C

Solubility **:** Soluble in water, chloroform, ethyl acetate.

3. P-HYDROXYBEZALDEHYDE:



Synonym :4-Hydroxy benzaldehyde

Molecular Formula : $C_7H_6O_2$

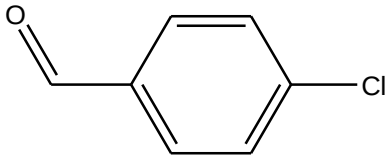
Formula weight : 122.12

Melting point : 112-116^{0}C

Boiling point :191^{0}C

Solubility : soluble in alcohol and water

4. 4-CHLOROBENZALDEHYDE:



Synonym : Parachlorobenzene carboxaldehyde

Molecular Formula : C_7H_5ClO

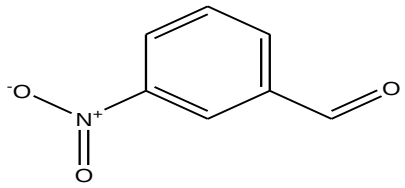
Formula weight : 140.57

Melting point : 46^{0}C

Boiling point : 213-216^{0}C

Solubility : very slightly soluble in alcohol.

4. 3-NITROBENZALDEHYDE:



Synonym : meta-benzaldehyde

Molecular Formula : **$C_7H_5NO_3$**

Formula weight : **151.12g/ mol^{-1}**

Melting point : **58.5 ^0C**

Boiling point : **164 ^0C**

Solubility : **Soluble in water**

CHARACTERIZATION

The purity of the synthesized compounds checked by TLC method and sharp melting point.

PHYSICAL EVALUATION:

1. The physical properties of the synthesized compounds are evaluated as follows
   - ❖ Colour
   - ❖ Nature
   - ❖ Solubility
   - ❖ Molecular weight
   - ❖ Molecular formula
   - ❖ Melting point
   - ❖ Boiling point.
2. Moreover the synthesized compounds are characterized by the following instrumental methods.

IR SPECTROMETRY

Infrared spectroscopy is one of most commonly used spectroscopic technique for identification of functional groups in molecules. IR spectroscopy is an important tool in the structural elucidation of organic compounds. In IR spectroscopy finger print region is used to compare the two compounds. Infrared spectrum shows percent transmittance versus frequency expressed as wave numbers.[69]

1. 3540-3300 cm-1 N-H Stretching Vibration
2. 3670-3230 cm-1 O-H Stretching Vibration
3. 1690-1630 cm-1 C=N Stretching Vibration
4. 2975-2840 cm-1 C-H Aliphatic Stretching Vibration

NMR SPECTROSCOPY

Nuclear Magnetic Resonance (NMR) spectroscopy is an important analytical technique used in the structural elucidation of organic molecules. It involves the interaction of the electromagnetic radiation and the proton of an nucleus of an atom when placed in an externally applied static magnetic field. NMR spectra provide the detailed information about a molecule's structure. The chemical shift is used to predict the number of protons with refers to DMSO as standard .The NMR spectra is recorded on 300 MH_Z BRUKER advance III NMR spectrometer. DMSO is used as a solvent.

1. Aromatic and hetero aromatic compounds 6-8.5 δ
2. Alcoholic hydroxyl protons 1-5.5 δ
3. Aldehyde protons 9-10 δ

HYPHENATED TECHNIQUE:

GC-MS:

Gas chromatography-mass spectrometry is a hyphenated technique, comprised of two analytical procedure in sequence, namely a gas chromatography (GC) which perform separation process and Mass spectroscopy (MS) which perform detection of separated fragments.

LC-MS:

LC-MS is a hyphenated technique, combining separation power of HPLC with the detection power of Mass Spectrometry.

BIOLOGICAL EVALUATION

Anti-tubercular Activity:

There are various assay methods available for the evaluation of new chemical entities against tuberculosis. They are as follows:

- ✓ MicroplateAlamar Blue Assay
- ✓ BACTEC Assay
- ✓ Luciferous Reporter Phage assay
- ✓ Resazurin Micro plate Assay(REMA)
- ✓ Broth Micro Dilution Assay
- ✓ Middle brook(7H 9,7H 10,7H 11) Agar Dilution Assay.
- ✓ Nitrate Reductase Assay

MICROPLATE ALAMAR BLUE ASSAY (MABA)[70]

- ❖ The anti-microbial activities of the synthesized compounds is determined by MABA method. The organism used in the studies is ***Mycobacteria tuberculosis*** (Vaccine strain, H37 RV strain): ATCC No- 27294.
- ❖ Alamar blue dye is used as an indicator for the determination of viable cells.

Principle:

MABA is an indirect colorimetric method for determining the MICs of TB drugs for strains of mycobacterium tuberculosis. In this assay, the redox indicator Alamar blue monitors the reducing environment of the living cells. It turns from blue to pink in the presence of mycobacterium growth.

Procedure:

1) The anti-mycobacterial activity of the compounds are to be assessed against M. tuberculosis using microplateAlamar blue assay (MABA).
2) This methodology is non- toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
3) Briefly, 200 ml of sterile deionized water is added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation.
4) The 96 wells plate received 100µl of the Middle brook 7H9 broth and serial dilution of compounds are placed directly on plate.
5) The final drug concentrations tested is made up to 100 to 0.2µg/ml.
6) Plates are covered and sealed with Para film and incubated at37^{o}C for five days.
7) After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to the plate and incubated for 24hrs.
8) A blue colour in the well is interpreted as no bacterial growth, and pink colour was scored as growth.

The MIC is defined as lowest drug concentration which prevents the colour change from blue to pink

ACUTE TOXICITY:

ACUTE ORAL TOXICITY STUDY:

Acute oral toxicity study (Limit Test) was designed as per the OECD guidelines (423).

Principles and purpose

The main purpose of acute toxicity is to evaluate the degree of toxicity in a quantitative and qualitative manner.

Experimental Animals

Six healthy adult Albino mice were weighing between 20-25g were selected for the study. For all the six animals food, but not water was withheld overnight prior to dosing.

Selection of dose levels and administration of dose:

Being synthetic molecules, the mortality was unlikely at the highest starting dose level (2000mg/kg/b.w). Hence a limit test one dose levels of 2000mg/kg/b.w was conducted in all animals as per the OECD guidelines (423).

Procedure:

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

CYTOTOXICITY[71]

MATERIAL:

Cell line: Vero (African green monkey kidney cells), from NCCS Pune, India.

DMEM, Fetal Bovine Serum (FBS), antibiotic–antimicotic solution from Thermoscientifc and SRB reagent from sigma Aldrich, USA. Tissue culture flasks, 96, well micro culture plates from Eppendorf, Germany.

METHODS:

Maintenance of cell lines

Vero (African green monkey kidney cells), cell line procured from NCCS Pune, India, procured from NCCS Pune were grown in 25 cm^2 tissue culture flasks containing DMEM

medium supplemented with 10% FBS, 1% L- glutamine and 1% antibiotic-antimicotic solutions at 37ºC in CO_2 incubator in an atmosphere of humidified 5% CO_2 and 95% air. The cells were maintained by routine sub culturing in 25 cm^2 tissue culture flasks.[71]

Sub culturing process of cell lines

- The culture media from the flasks containing monolayer culture was aspirated and washed with sterile phosphate buffered saline (PBS).
- To the flasks, 1 ml of 0.2% trypsin-EDTA solution was added and after few seconds it was aspirated and flask was kept in incubator 2-3 min. for detachment.
- The flasks were removed from the incubator and gently tapped to detach all the adhering cells. The cell detachment was confirmed by observing under an inverted microscope (Nikon Eclipse TE 2000-5, Japan).
- Once the cells were completely detached from the flasks, 2-3 ml of DMEM media containing 10% FBS was added and mixed well.
- From the stock cell suspension, 1 x 10^5 viable cells/ml suspended in media were seeded in 25cm^2 tissue culture flask containing about 4ml of fresh media and incubated until the flasks attained 60-70% confluence.

Trypsinization

To obtain a single cell suspension from a monolayer culture, cells were dislodged form the culture flasks by trypsinization.

- From a 60-70% confluent flask, the culture media was aspirated out using a micropipette.
- Cells were washed with 3 ml of PBS to remove trace amount of media.
- To each culture flask 1 ml of trypsin-EDTA was added and after few seconds it was aspirated and the flask was kept in the incubator for 3-4 min for cell detachment
- Culture flasks were observed under an inverted microscope (Nikon Eclipse, Japan) to ensure that cells were completely dislodged.

- Trypsin activity was stopped by adding 2-3ml media containing 10% FBS.

Seeding:

Exponentially growing cell lines were harvested from 25cm^2 Tissue culture flask and a stock cell suspension (5X10^6 cell/ml) was prepared.

- A 96-well flat bottom tissue culture plate was seeded with 5 x10^3 cells in 0.1 ml of DMEM medium supplemented with 10% FBS and allowed to attach for 24hrs.

Preparation of drug dilutions: (serial dilution)

- 50mg/ml stock solution was prepared using 100% DMSO solution. From this stock solution various final concentrations (viz. 62.5, 125, 250 and 500 µg/ml) of test compound solution was prepared as follows:
- 500 µg/ml: 10 µL sol. was taken from stock and to this 990 µl media was added.
- 250 µg/ml: From 500 µg/ml 500 µl was taken and diluted with 500 µl with media.
- 125 µg/ml: From 250 µg/ml 500 µl was taken and diluted with 500 µl with media.
- 62.5 µg/ml: From 125 µg/ml 500 µl was taken and diluted with 500 µl with media
- After 24 hrs of incubation, cells were treated with 100 µl of test solutions from respective above stocks and the cells were incubated for 48 hrs.
- The cells in the control group received only the medium containing the 0.5, 0.1 % DMSO.
- Each treatment was performed in triplicates.

SRB Assay:

Principle:

Sulforhodamine B (SRB) assay was developed in 1990 is one of the most widely used methods for *in vitro* cytotoxicity screening. The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA).

SRB is a bright-pink amino xanthene dye with two sulfonic groups that binds to basic amino-acid residues under mild acidic conditions and dissociate under basic conditions. As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass.

Reagents

- 10% (wt/vol) TCA
- 1% (vol/vol) acetic acid
- 0.057% (wt/vol) SRB (Sigma, cat. no. 86183) in 1% (vol/vol) acetic acid
- 10 mM unbuffered Tris base solution

Cell fixation and staining

- 100 µl ice cold 10% (wt/vol) TCA was gently added to each well and the plates at 4 °C for 1 h.
- The plates were washed four times under slow-running tap water and excess water was removed by air drying the plates at room temperature.
- 100 µl 0.057% (W/V) SRB was added to the wells and incubated for 30 minutes at room temperature in dark.
- The plates were then washed with 1% acetic acid to remove the unbound dye. The plates were air dried at room temperature.
- 200 µl of Tris base (10mM) was added to the wells and plates were places on shaker for 10 min.
- Optical density was read at 540 nm in a ELISA plate reader. Percentage cell death was calculated (absorbance of control wells- absorbance of test wells / absorbance of control wells) x 100.

IC50 was calculated from the % cell death using GraphPad Prism 7 software.

RESULTS AND DISCUSSION

RESULT AND DISCUSSION:

PREDICTION OF DRUG LIKENESS:

The designed and docked molecules were screened insilico using **Molinspiration cheminformatics®** to evaluate drug likeness. Molinspiration® is used to calculate important physicochemical properties of molecules such as log P, polar surface area, number of hydrogen bond donors and acceptors.

Variants:

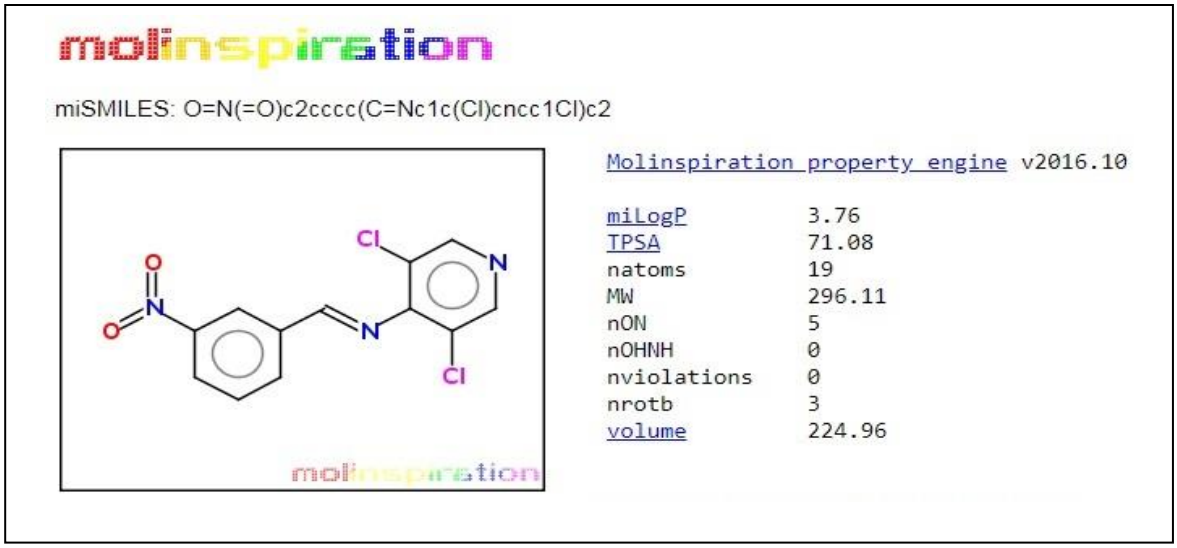
In an attempt to improve the predictions of druglikeness, the rules have spawned many extensions, for example the following:

- Partition coefficient log *P* in −0.4 to +5.6 range
- Molar refractivity from 40 to 130180
- Molecular weight from to 500
- Number of atoms from 20 to 70 (includes H-bond donors [e.g. OHs and NHs] and H-bond acceptors

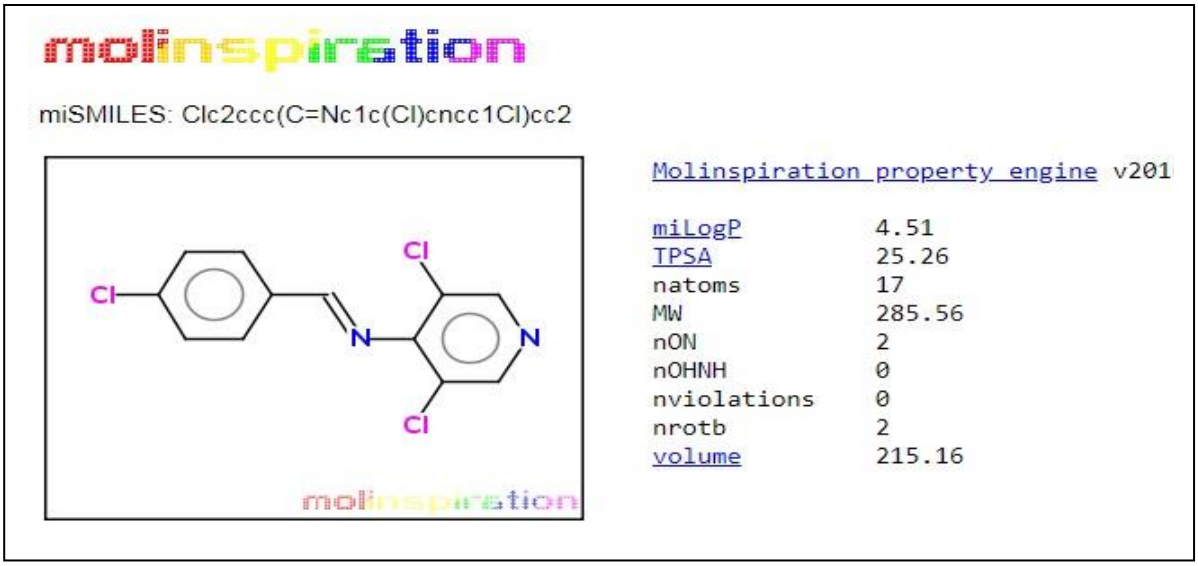
PREDICTION OF DRUG LIKENESS:

The snapshot of the molecules in drug likeness is presented below for the chosen compounds. All the compounds were compare with Lipinski's rule and chosen for synthesis.

COMPOUND NAME: DCP1



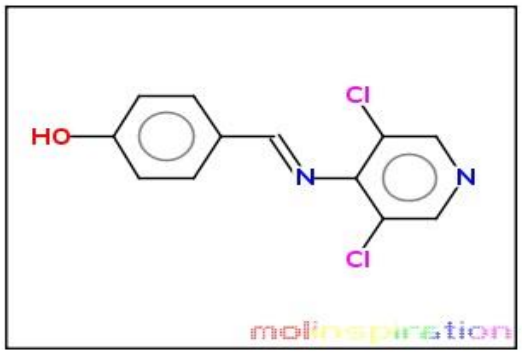
COMPOUND NAME: DCP2



COMPOUND NAME: DCP3

molinspiration

miSMILES: Oc2ccc(C=Nc1c(Cl)cncc1Cl)cc2



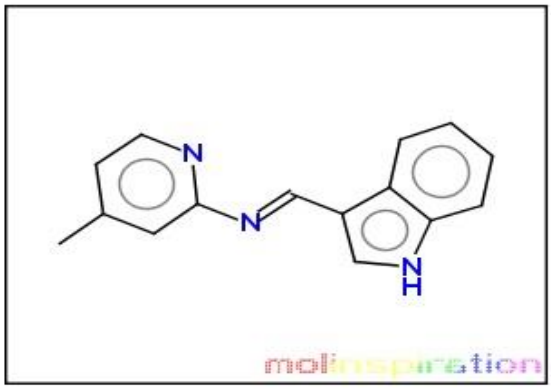
Molinspiration property engine v2016.10

miLogP	3.35
TPSA	45.48
natoms	17
MW	267.12
nON	3
nOHNH	1
nviolations	0
nrotb	2
volume	209.65

COMPOUND NAME: PIA

molinspiration

miSMILES: Cc3ccnc(N=Cc1c[nH]c2ccccc12)c3



Molinspiration property engine v201

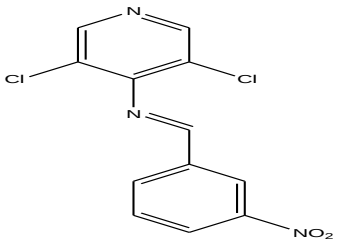
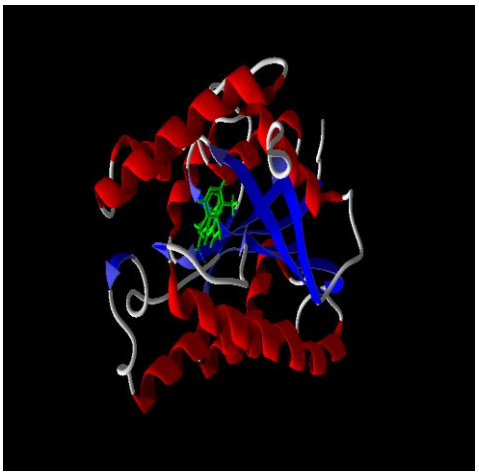
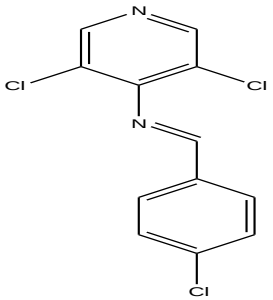
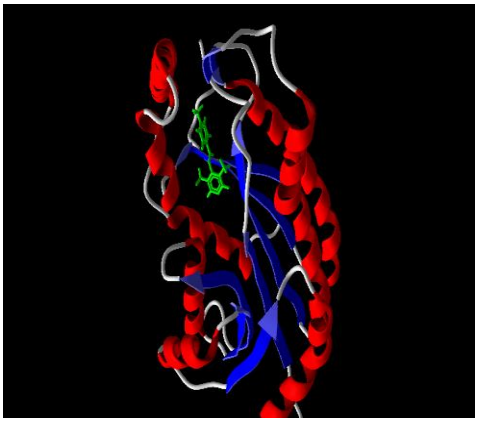
miLogP	3.12
TPSA	41.05
natoms	18
MW	235.29
nON	3
nOHNH	1
nviolations	0
nrotb	2
volume	220.09

DOCKING STUDIES:

ACTIVITY PREDICTION:

More than 200 scaffolds were docked against the MTB enzyme Inh A (Enolyl Acyl Carrier Protein) (2h9i) by using Autodock® 4.2.5.1 software. The result of the docking and the view for different compound is presented below.

Table 1: Docking Score And View Using Autodock® 4.2.5.1

Compound code	Structure	Docking Score	Docking view
DCP1		**-5.58 Kcal/mol**	
DCP2		**-7.02 Kcal/mol**	

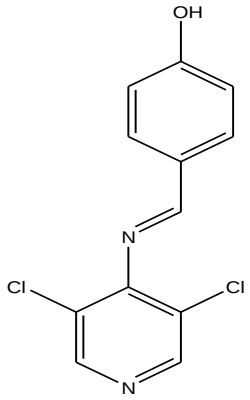
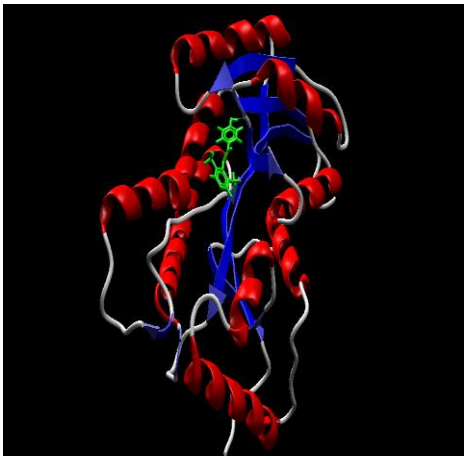
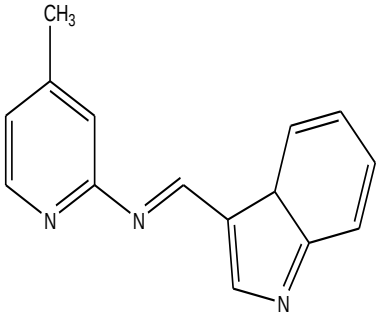
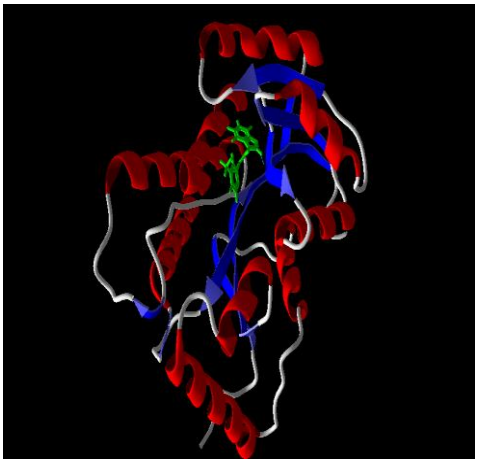
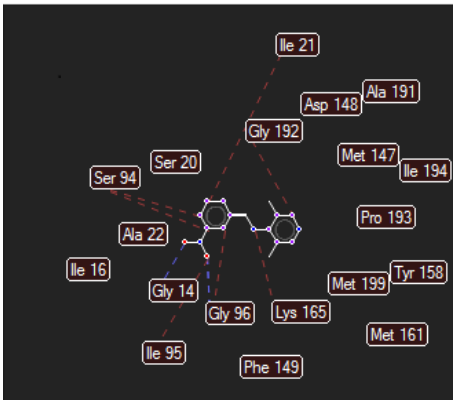
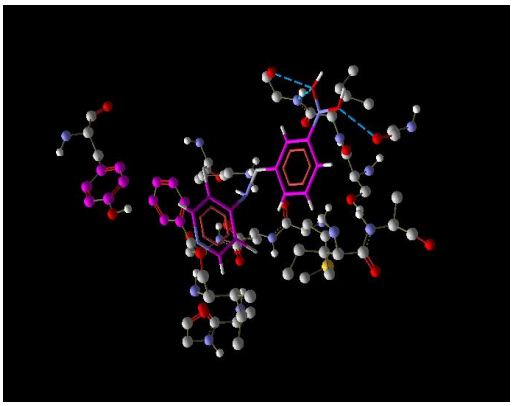
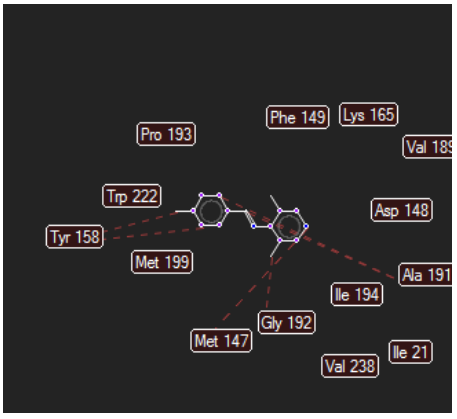
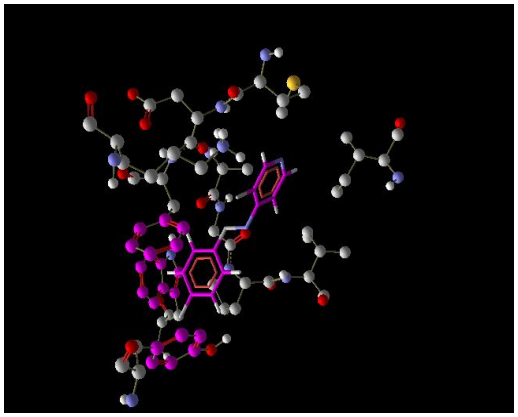
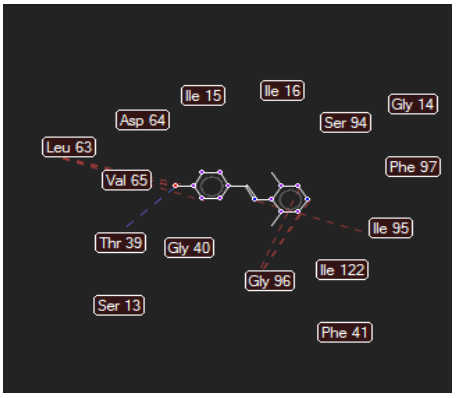
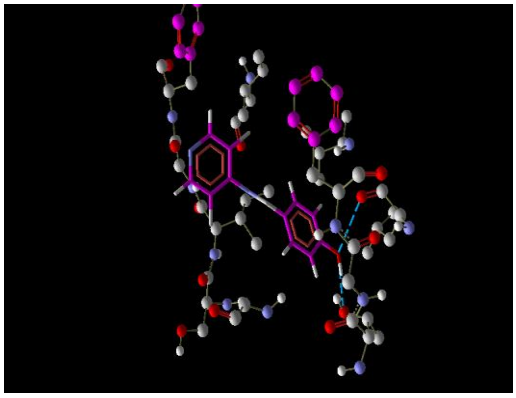
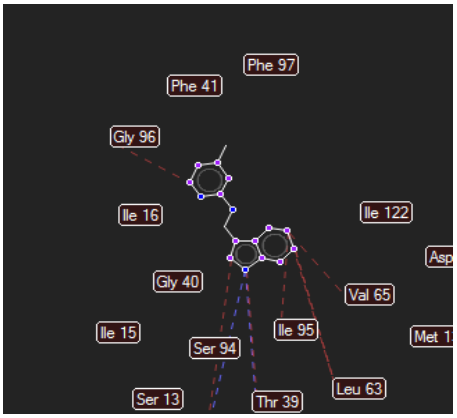
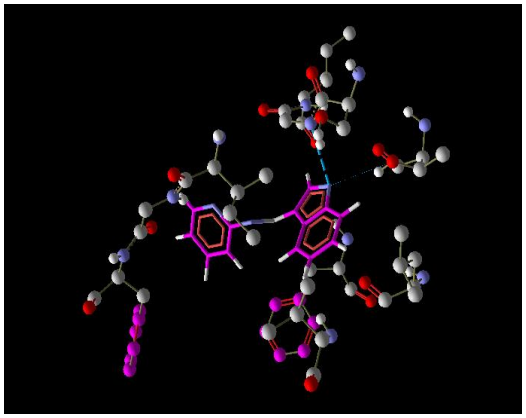
DCP3		**-6.53 Kcal/mol**	
PIA		**-7.0 Kcal/mol**	

Table 2 : 2h9i Interaction With Ligand

Compound code	Interaction with Aminoacid	Hydrogen bond Interaction
DCP1		
DCP2		
DCP3		

PIA		

Toxicity prediction:

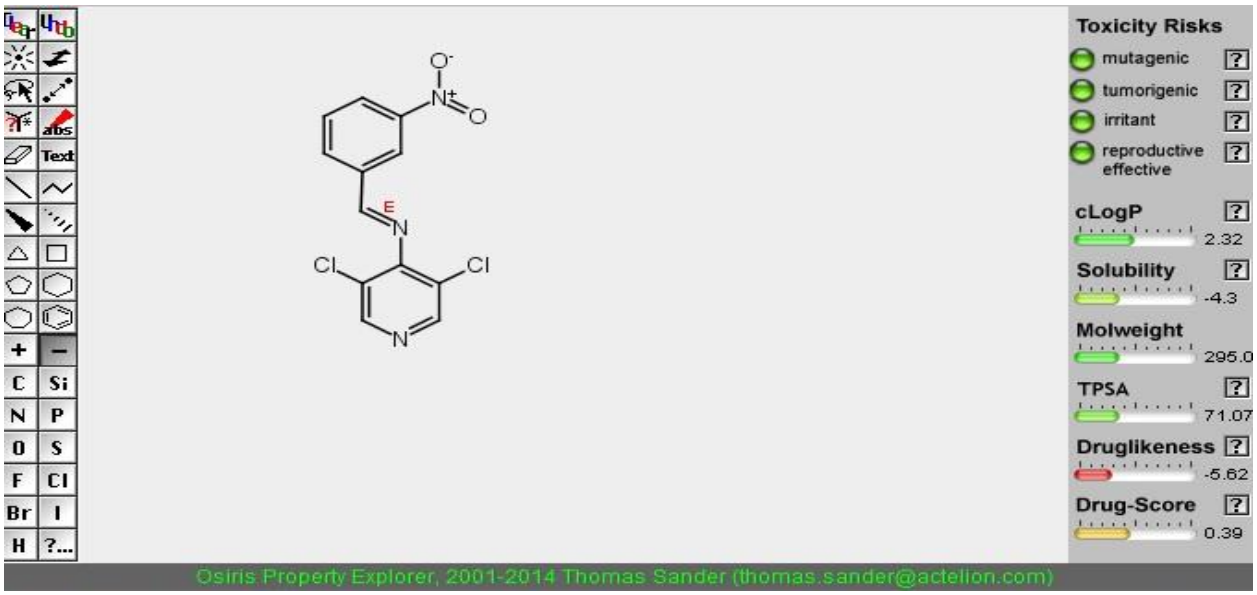
OSIRIS® Property Explorer the online software of Thomas Sander, Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, and 4123 Allschwil, Switzerland. This applet predicts physico-chemical properties and detects potential toxicity risks for any drawn chemical structure in real time.

The Prediction result was indicated by color coded. For predicting properties of a chemical compound just draw its structure and *Property Explorer* will start calculating properties as soon as a chemical structure is valid. Charges should be balanced and atom valances not exceeded. Properties with high risks of **undesired effects** like mutagenicity or a poor intestinal absorption are shown in **red.** Whereas **green** color indicates the **drug conform** behaviour.

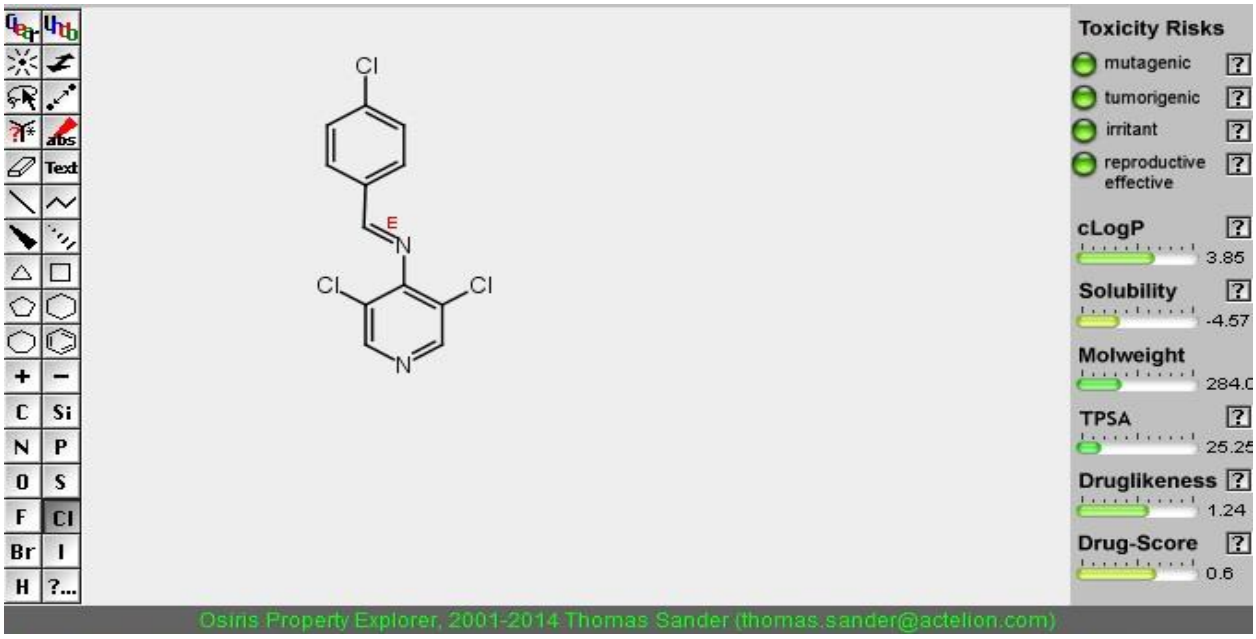
Table 3 : Prediction Of Toxicity Using Osiris® Property Explorer

TOXICITY	COMPOUND CODE			
	DCP1	DCP2	DCP3	PIA
MUTAGENIC	_ _ _	_ _ _	_ _ _	_ _ _
TUMOROGENIC	_ _ _	_ _ _	_ _ _	_ _ _
IRRITANT	_ _ _	_ _ _	_ _ _	_ _ _
REPRODUCTIVE EFFECT	_ _ _	_ _ _	_ _ _	_ _ _

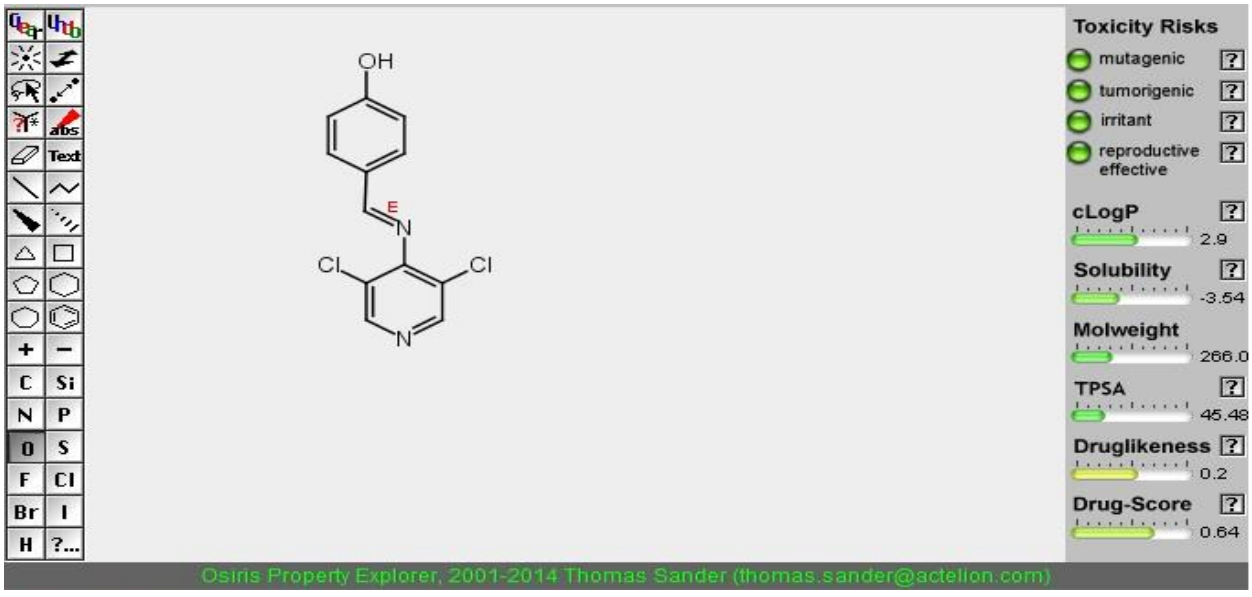
SAMPLE CODE :DCP1



SAMPLE CODE :DCP2



SAMPLE CODE :DCP3



SAMPLE CODE: PIA

RESULTS OF SYNTHETIC EFFORTS:

The selected **4** compounds were synthesized and recrystallised. Then the synthesized compounds were evaluated for their purity through melting point determination and TLC was carried out for the determination of absence of present compounds on other new compounds.

1. 3,5-dichloro 4-amino pyridine
2. 2-amino 4-methyl pyridine

TABLE:4 RESULTS OF SYNTHETIC EFFORTS:

S.NO	Compound code	Molecular weight	Percentage Yield	Melting point
1	**DCP1**	295.06 g/mole	76%	78^0 C
2	**DCP2**	286.88 g/mole	78%	114^0 C
3	**DCP3**	266.91 g/mole	62%	104^0 C
4	**PIA**	237.55 g/mole	67%	120^0 C

The Rf value of the synthesized compounds are distinguished from the Rf value of the reactants. Hence it is concluded that the reaction was completed.

The synthesized compounds were subjected to purification by recrystallization and TLC. The melting point of the synthesized compounds was founded. The characterization was carried out using sophisticated instruments like IR, NMR, and Mass spectroscopy and characteristic properties through the aid of computer software.

Infrared Absorption Spectroscopy:

The IR spectrums of the synthesized compounds were inspected for presence of the new functional group and absence of the functional group which induced the changes in the chemical reaction.

H^1 NMR Spectroscopy:

Proton NMR spectroscopy help us to study the number of equivalent protons and their environment thereby we can ascertain the structure of molecule. The positions of the signals help us to know the nature of protons viz, aromatic, heteroaromatic, aliphatic, vinyl C-H groups.The H^1 NMR spectral data of all the synthesized compounds are in conformity with the structure assigned. A singlet at 11.02-11.35 was observed for all compounds confirming the presence of N-H proton. All the compounds shows the multiplet and doublet signals for the presence of aromatic protons between (6.2-7.4)heteroaromatic protons between (7.2-7.8) δppm.

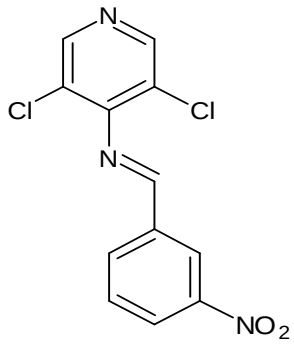
Table no:5 Molecular Weight of Synthesized compounds:

NAME OF COMPOUNDS	CALCULATED MASS	ACTUAL MASS
DCP1	295.06g/mole	296.11g/mole
DCP2	286.88g/mole	285.55g/mole
DCP3	266.91g/mole	267.11g/mole
PIA	237.55g/mole	235.28g/mole

PRODUCT PROFILE:

CODE: DCP1

IUPACNAME: (*E*)-*N*-(3,5-dichloropyridin-4-yl)-1-(3 nitrophenyl)methanimine



Molecular Formula = $C_{12}H_7Cl_2N_3O_2$

Formula Weight = 296.10888

Composition = C(48.67%) H(2.38%) Cl(23.95%) N(14.19%) O(10.81%)

Molar Refractivity = 73.71 ± 0.5 cm^3

Molar Volume = 202.2 ± 7.0 cm^3

Parachor = 550.7 ± 8.0 cm^3

Index of Refraction = 1.649 ± 0.05

Surface Tension = 55.0 ± 7.0 dyne/cm

Density = 1.46 ± 0.1 g/cm^3

Polarizability = 29.22 ± 0.5 $10^{-24}cm^3$

Monoisotopic Mass = 294.991532 Da

Nominal Mass = 295 Da

Average Mass = 296.1089 Da

IR SPECTRUM DCP1:

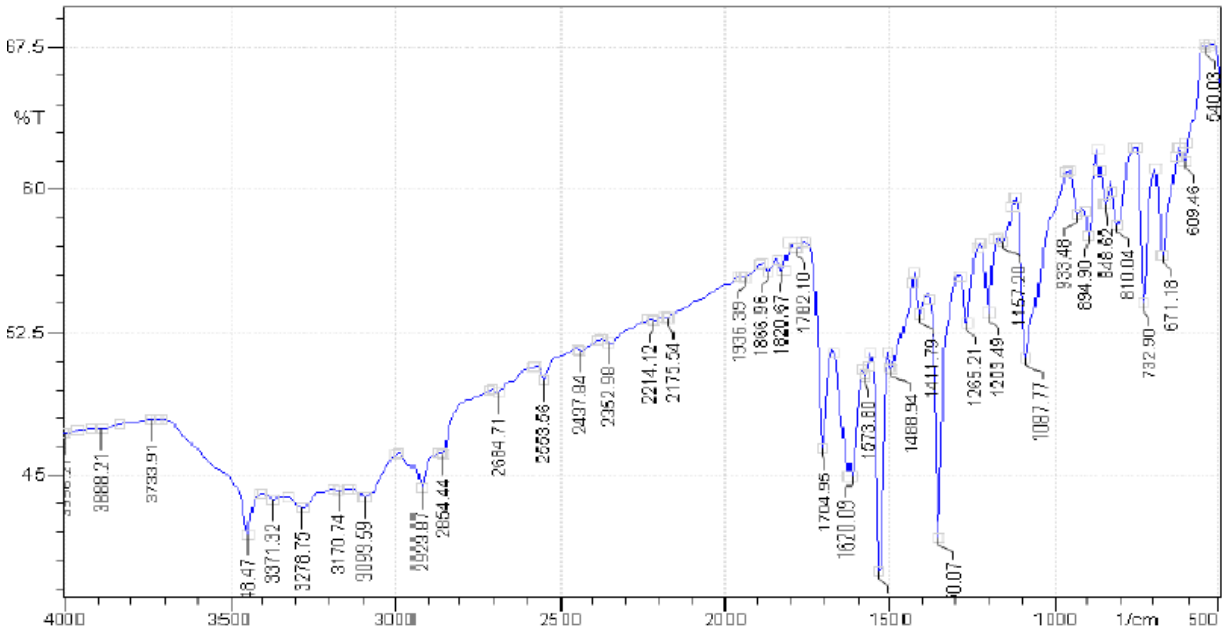
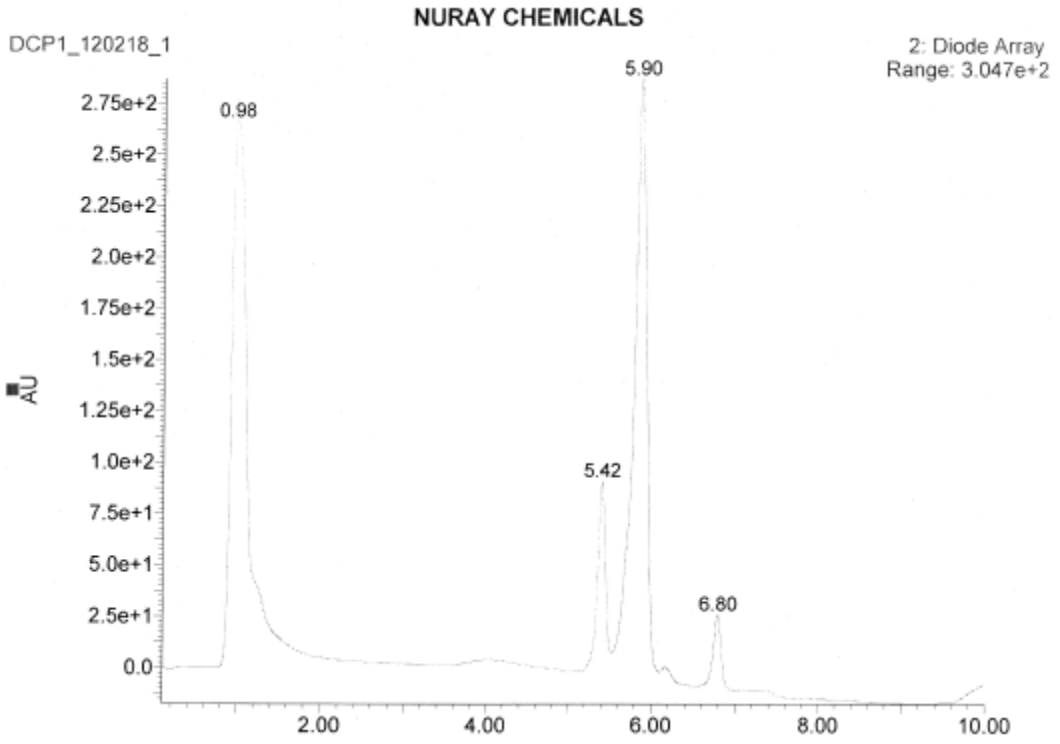


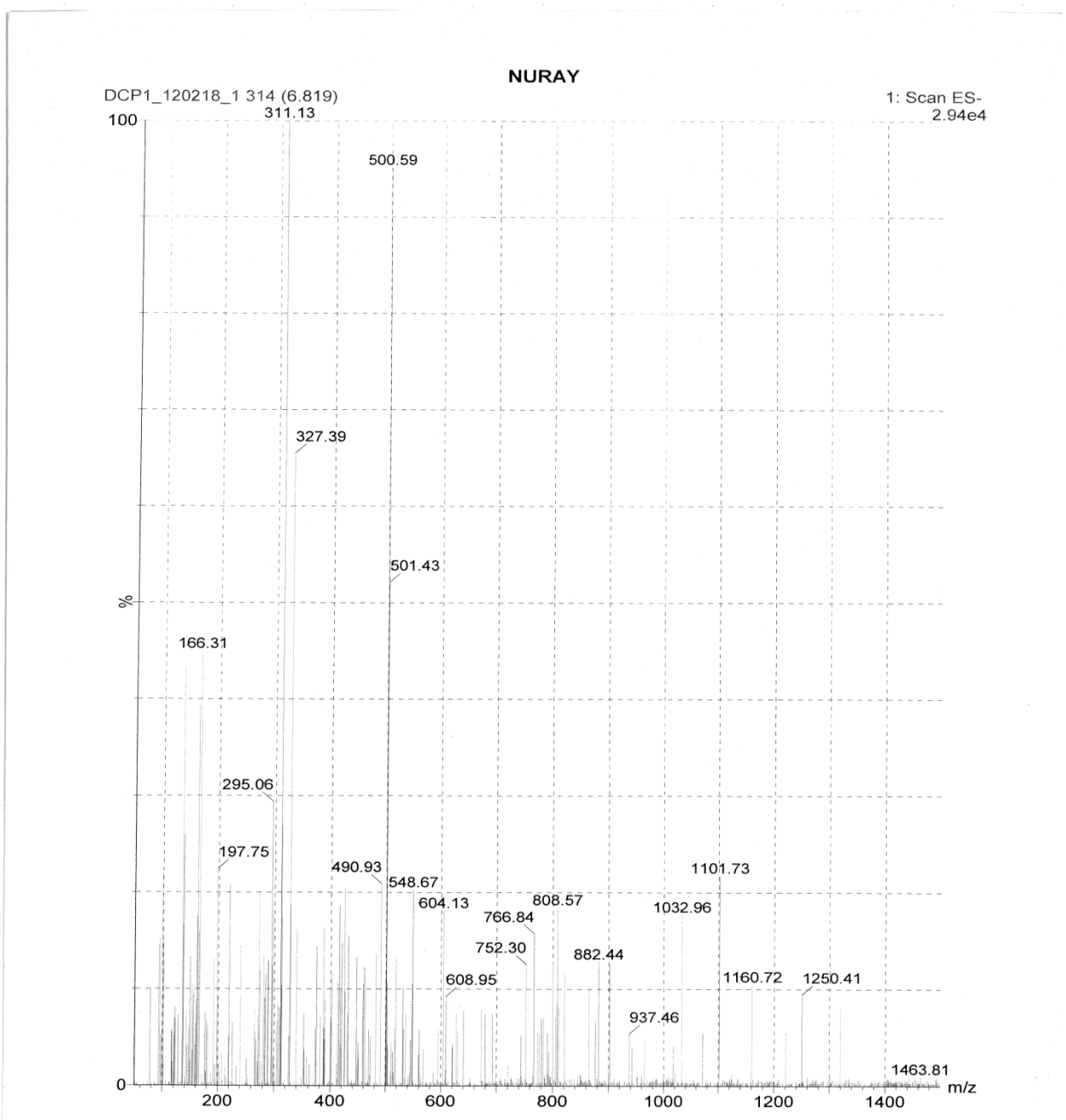
Table No6: INTERPRETATION OF IR

S.No	Wave number (cm^{-1})	Functional group
1.	2929.87	C-H Stretching
2.	732.90	C-Cl Stretching
3.	1629.09	C=N Stretching
4	1504.37	-NO Streching

LC-MS CHROMATOGRAM: DCP1



LC-MS SPECTRUM: DCP1



NMR SPECTRUM: DCP1

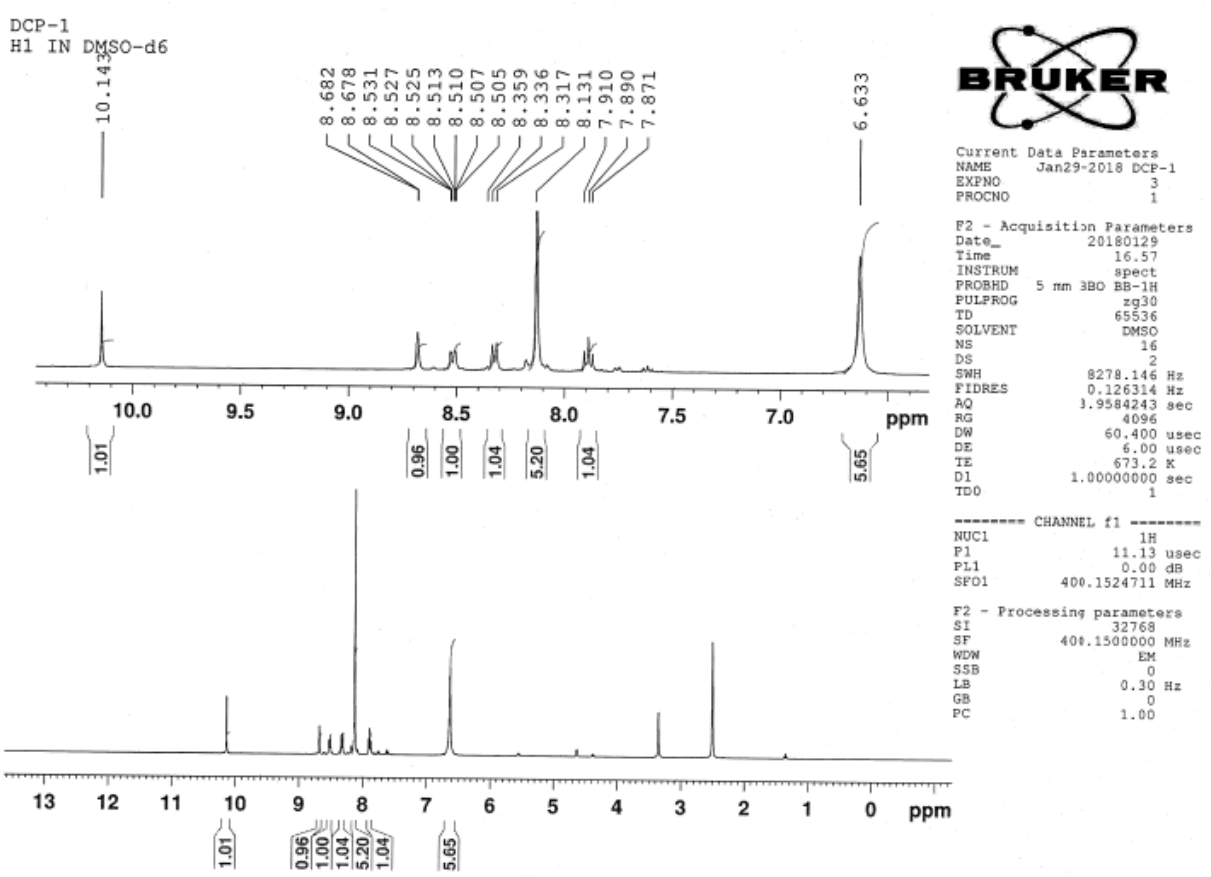


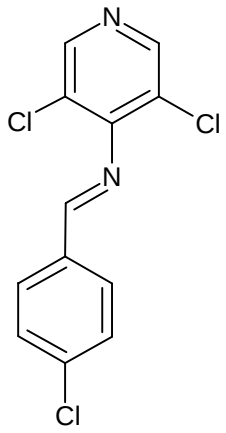
Table No 7: INTERPRETATION OF NMR

S.No	δ VALUE (PPM)	NATURE OF PEAK	NUMBER OF PROTONS
1	δ6.8	Multiplet	5 Protons
2	δ7.9	Multiplet	5 Protons
3	δ10.2	Singlet	1 Protons

PRODUCT PROFILE:

CODE:DCP2

IUPAC NAME: (*E*)-1-(4-chlorophenyl)-*N*-(3,5-dichloropyridin-4 yl)methanimine



Molecular Formula = $C_{12}H_7Cl_3N_2$

Formula Weight = 285.55638

Composition = C(50.47%) H(2.47%) Cl(37.25%) N(9.81%)

Molar Refractivity = 72.65 ± 0.5 cm^3

Molar Volume = 206.1 ± 7.0 cm^3

Parachor = 534.1 ± 8.0 cm^3

Index of Refraction = 1.622 ± 0.05

Surface Tension = 45.0 ± 7.0 dyne/cm

Density = 1.38 ± 0.1 g/cm^3

Polarizability = 28.80 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM DCP2:

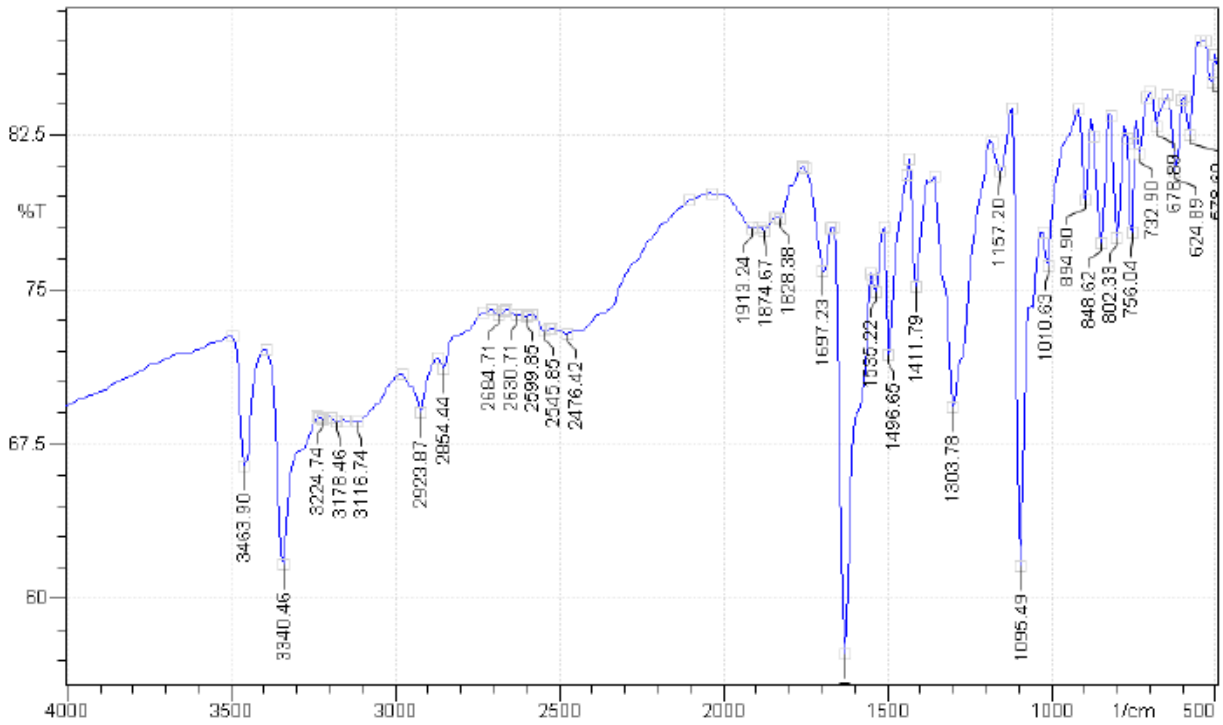
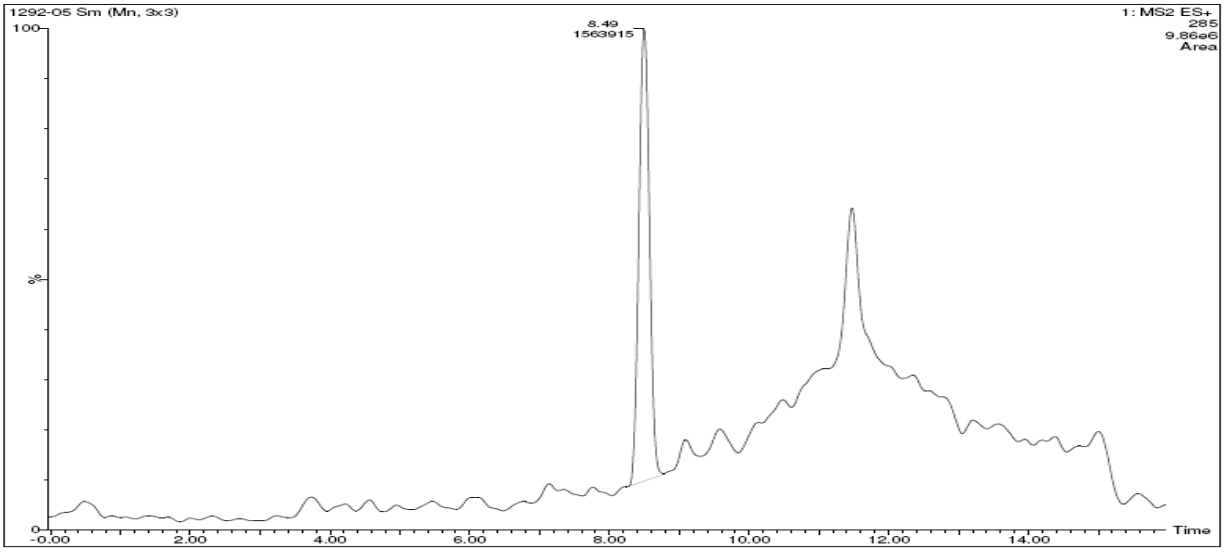


Table No:8 INTERPRETATION OF IR:

S.No	Wave number (cm^{-1})	Functional groups
1.	732.90	C-Cl Stretching
2.	1550.65	C=N Stretching
3.	3240.17	C-H Stretching

LC-MS CHROMATOGRAM:DCP2



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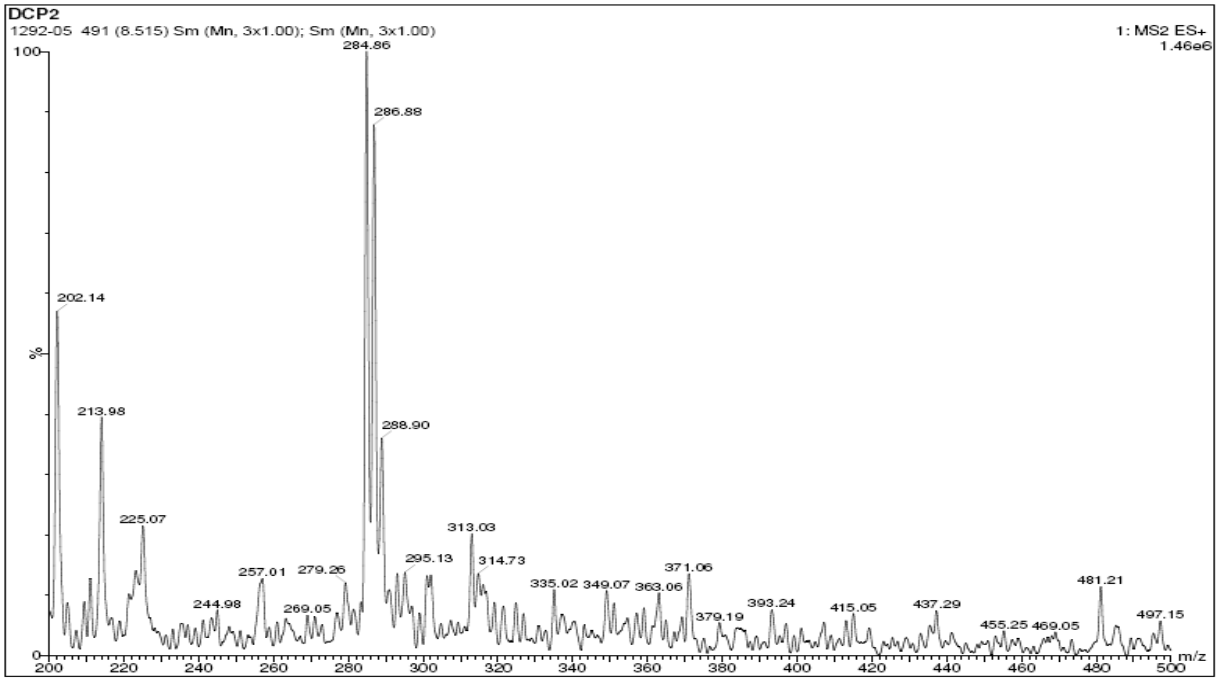


NMR SPECTRUM DCP2:

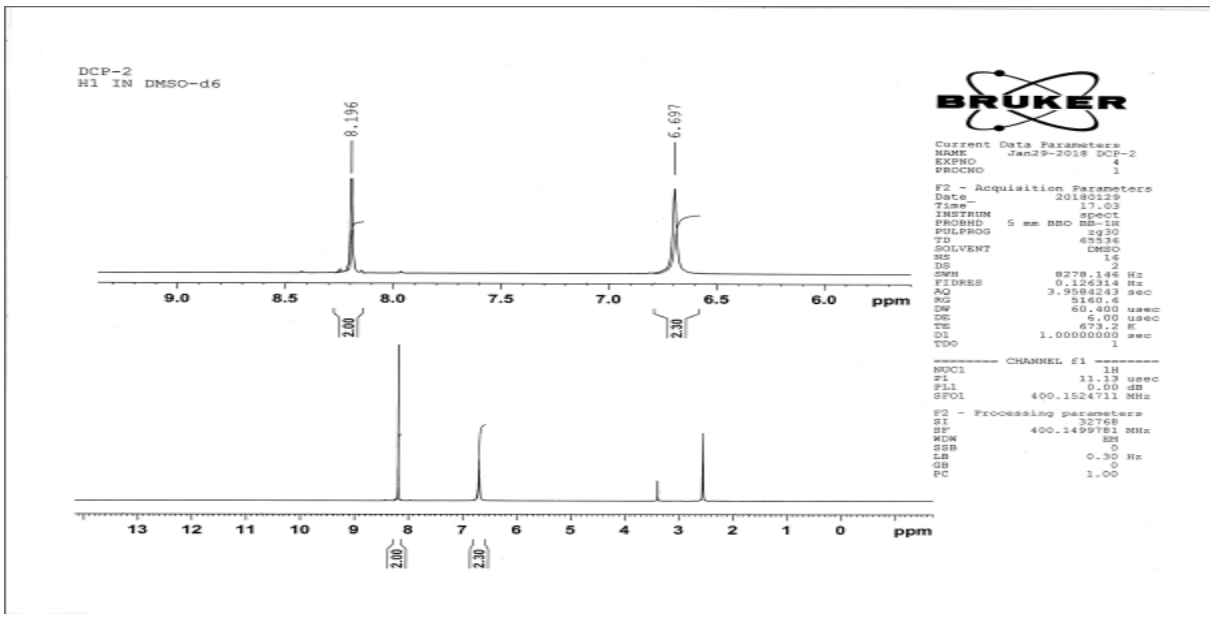


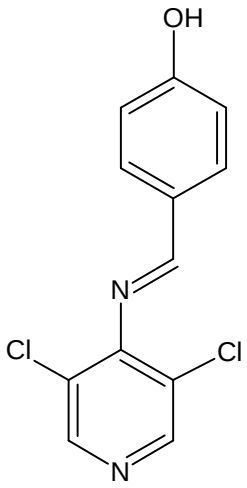
Table No:9 INTERPRETATION OF NMR:

S.No	δ VALUE (PPM)	NATURE OF PEAK	NUMBER OF PROTONS
1	δ6.8	Doublet	2 Protons
2	δ8.2	Doublet	2 Protons

CODE :DCP3

IUPAC NAME

4-{(*E*)-[(3,5-dichloropyridin-4-yl)imino]methyl}phenol



Molecular Formula = $C_{12}H_8Cl_2N_2O$

Formula Weight = 267.11072

Composition = C(53.96%) H(3.02%) Cl(26.55%) N(10.49%) O(5.99%)

Molar Refractivity = 68.91 ± 0.5 cm^3

Molar Volume = 194.1 ± 7.0 cm^3

Parachor = 510.9 ± 8.0 cm^3

Index of Refraction = 1.628 ± 0.05

Surface Tension = 48.0 ± 7.0 dyne/cm

Density = 1.37 ± 0.1 g/cm^3

Dielectric Constant = Not available

IR SPECTRUM DCP3:

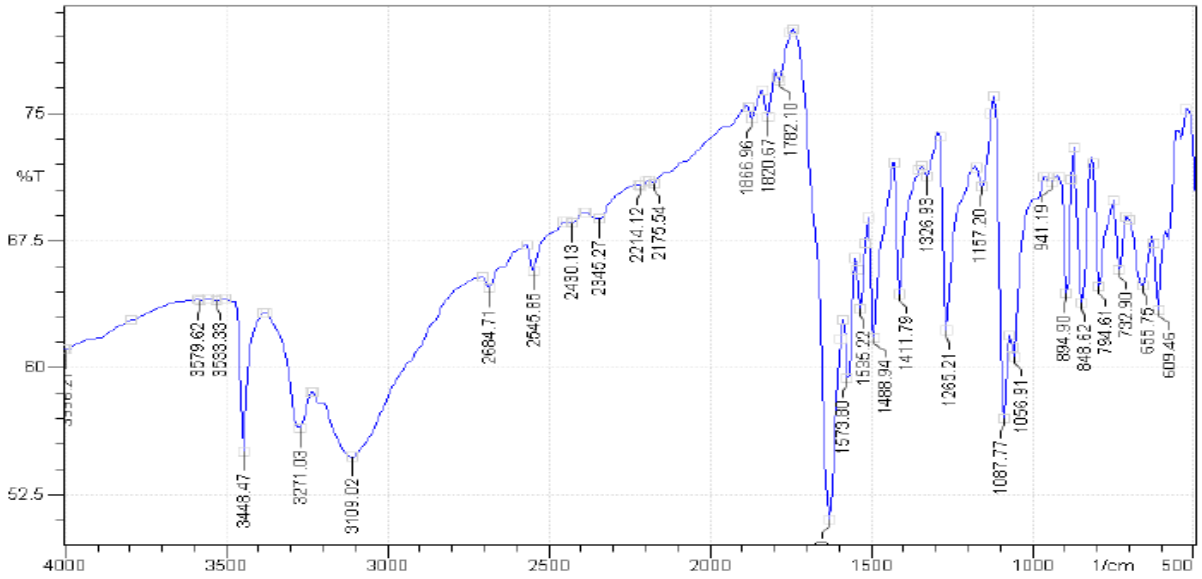
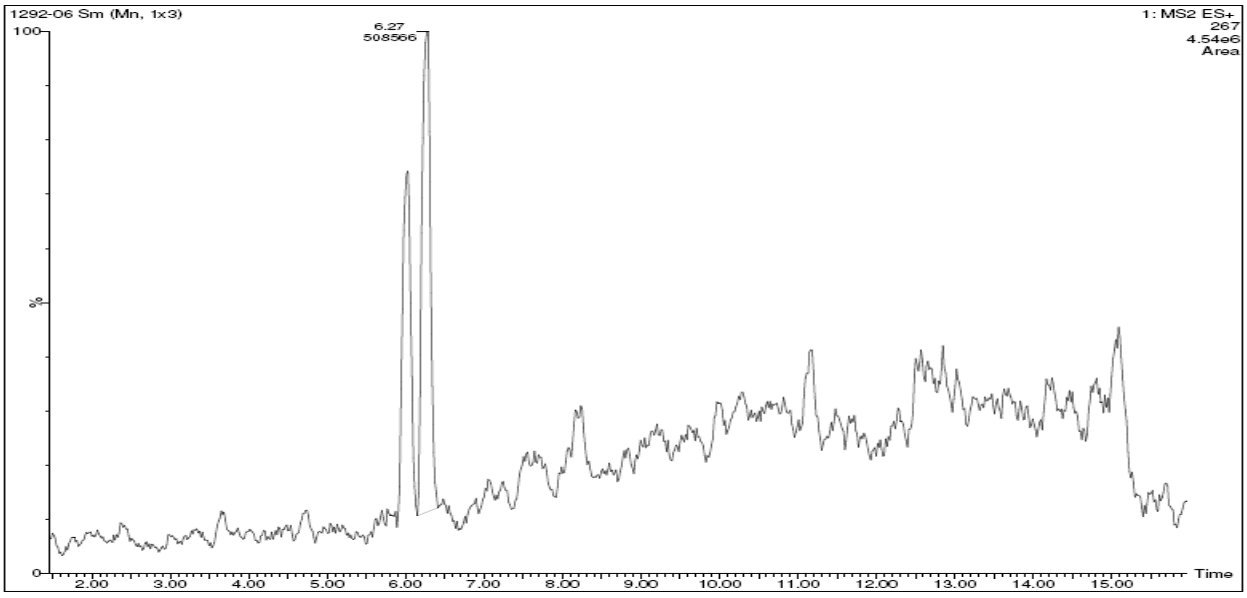


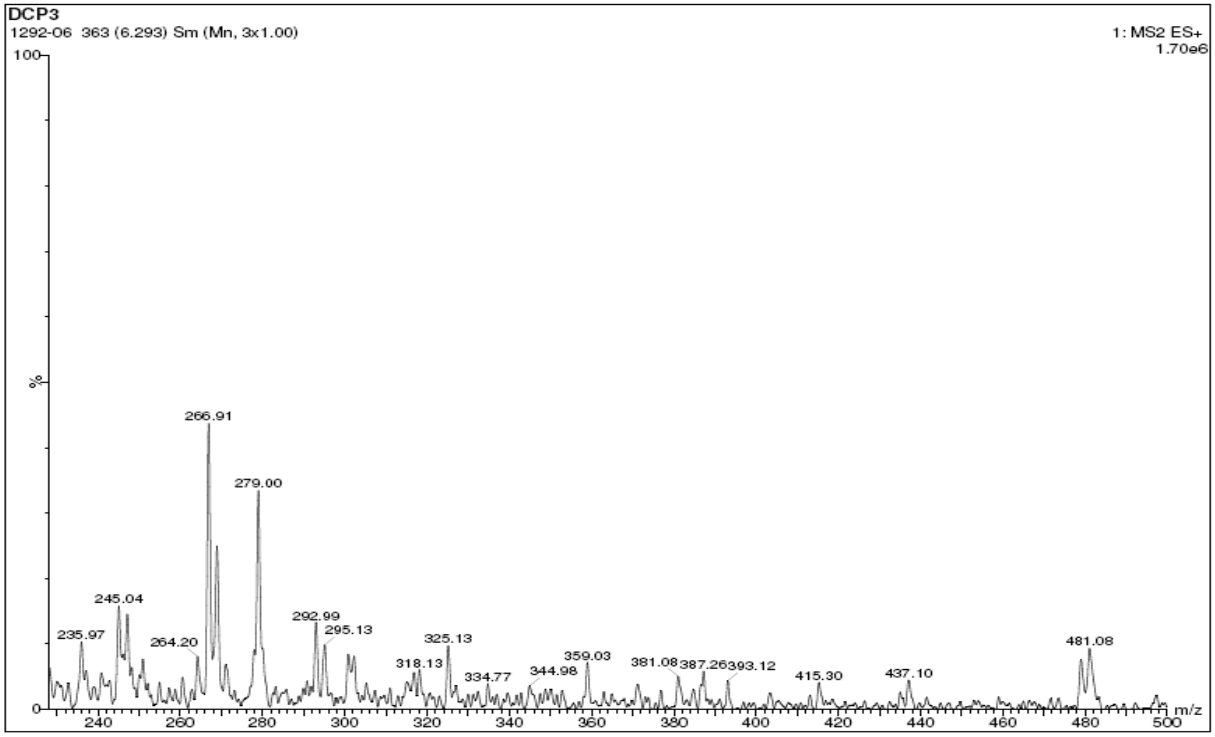
Table No:10 INTERPRETATION OF IR:

S.No	Wave number (cm^{-1})	Functional groups
1	3448.47	-OH Stretching
2	1172.63	-C-O Stretching
3	1550.65	-C=N Stretching
4	732.90	-C-Cl Stretching

LC-MS CHROMATOGRAM DCP3:



LC-MS SPECTRUM DCP3:



NMR:DCP3

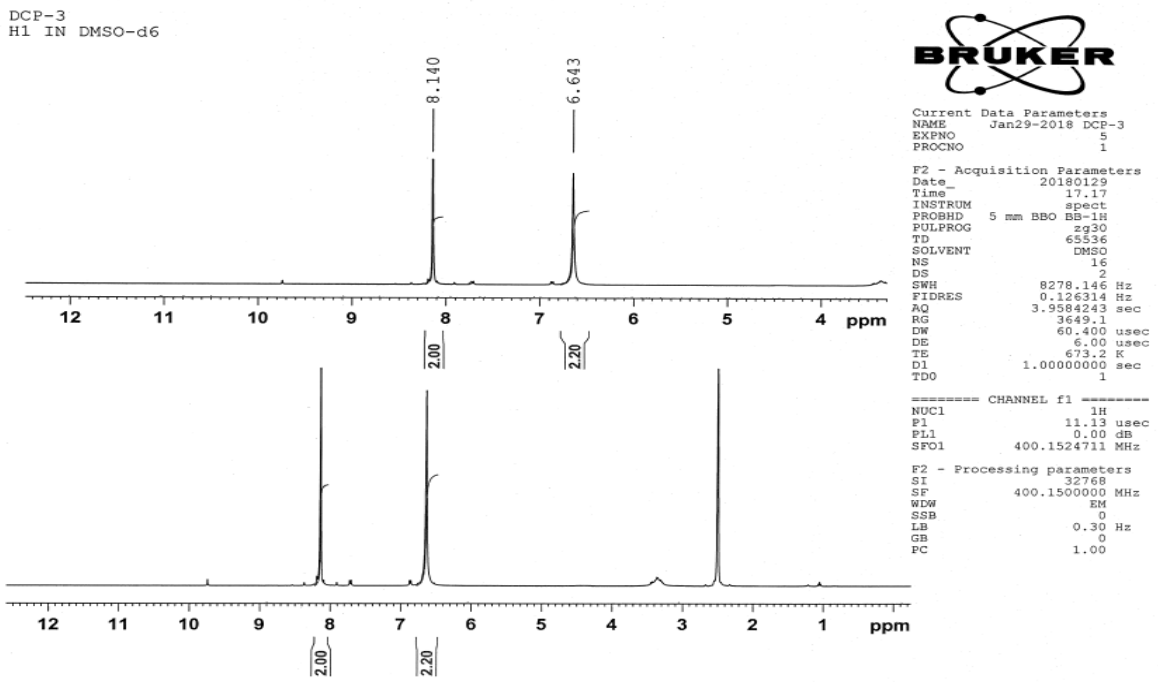


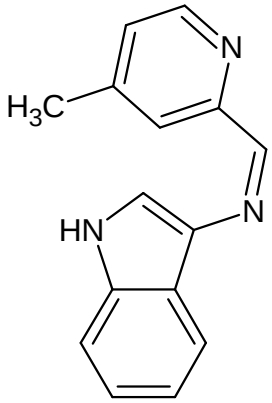
Table No:11 INTERPRETATION OF NMR:

S.No	δ VALUE (PPM)	NATURE OF PEAK	NUMBER OF PROTONS
1.	δ6.8	Doublet	2 Protons
2.	δ8.2	Doublet	2 Protons

PRODUCT DETAILS

CODE:PIA

IUPAC NAME: (*Z*)-*N*-(1*H*-indol-3-yl)-1-(4-methylpyridin-2-yl)methanimine



Molecular Formula = $C_{15}H_{13}N_3$

Formula Weight = 235.28382

Composition = C(76.57%) H(5.57%) N(17.86%)

Molar Refractivity = 73.00 ± 0.5 cm^3

Molar Volume = 201.8 ± 7.0 cm^3

Parachor = 520.8 ± 8.0 cm^3

Index of Refraction = 1.643 ± 0.05

Surface Tension = 44.3 ± 7.0 dyne/cm

Density = 1.16 ± 0.1 g/cm^3

Dielectric Constant = Not available

Polarizability = 28.94 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM: PIA

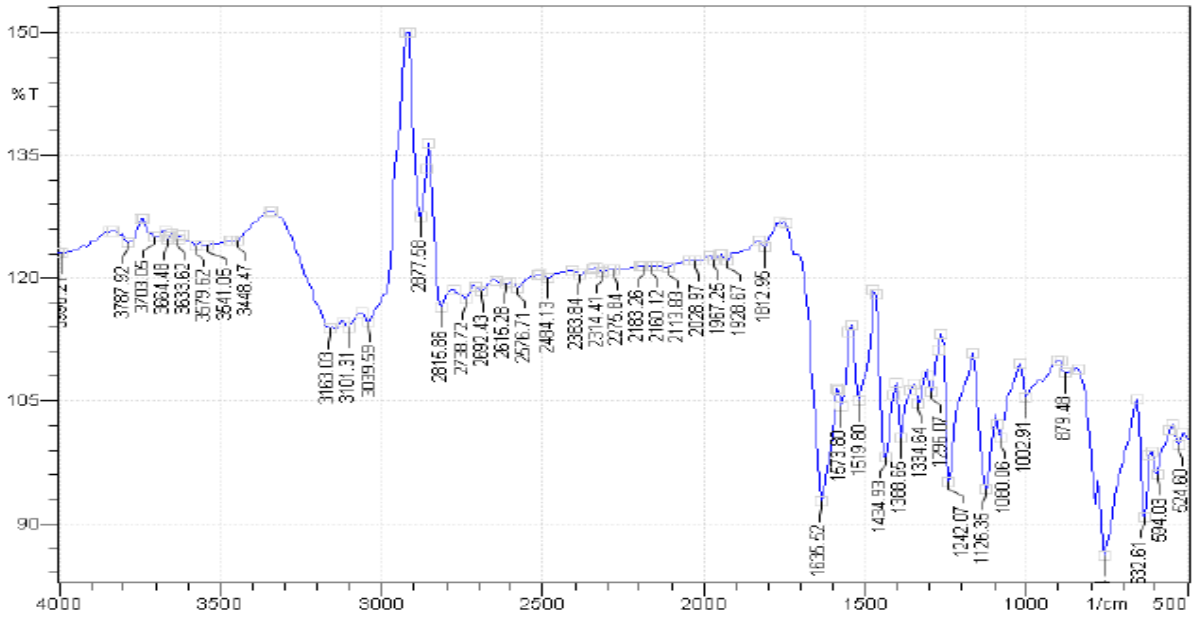
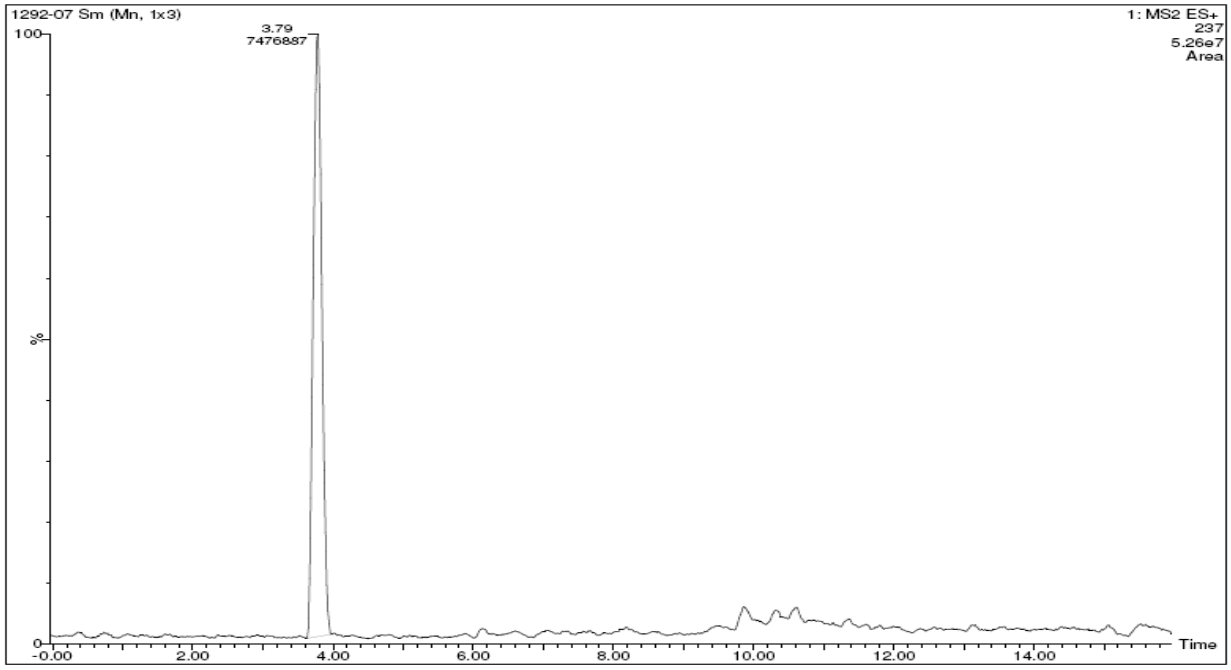


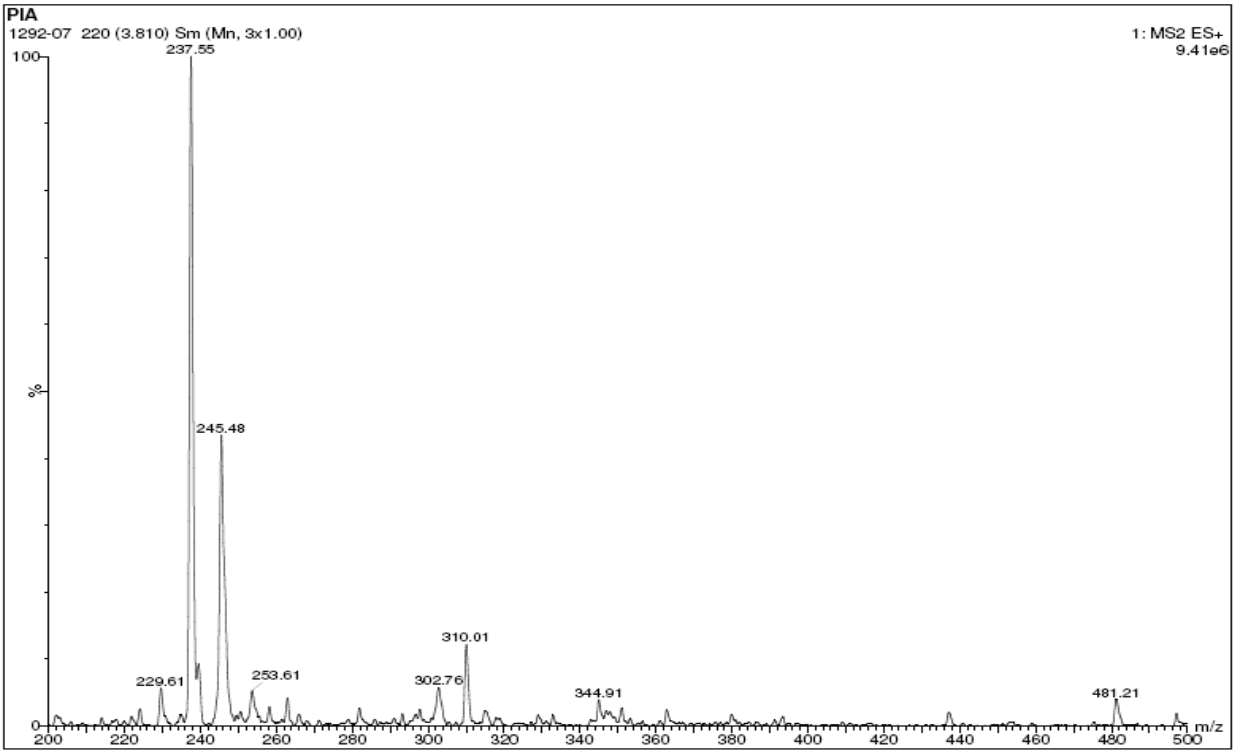
Table No:12 INTERPRETATION OF IR

S.No	Wave number (cm^{-1})	Functional groups
1	3448.47	-NH Stretching
2	1589.3	-C=N Stretching
3	1473.51	-C-H Stretching

LC-MS CHROMATOGRAM PIA:



LC-MS SPECTRUM PIA:



NMR SPECTRUM PIA:

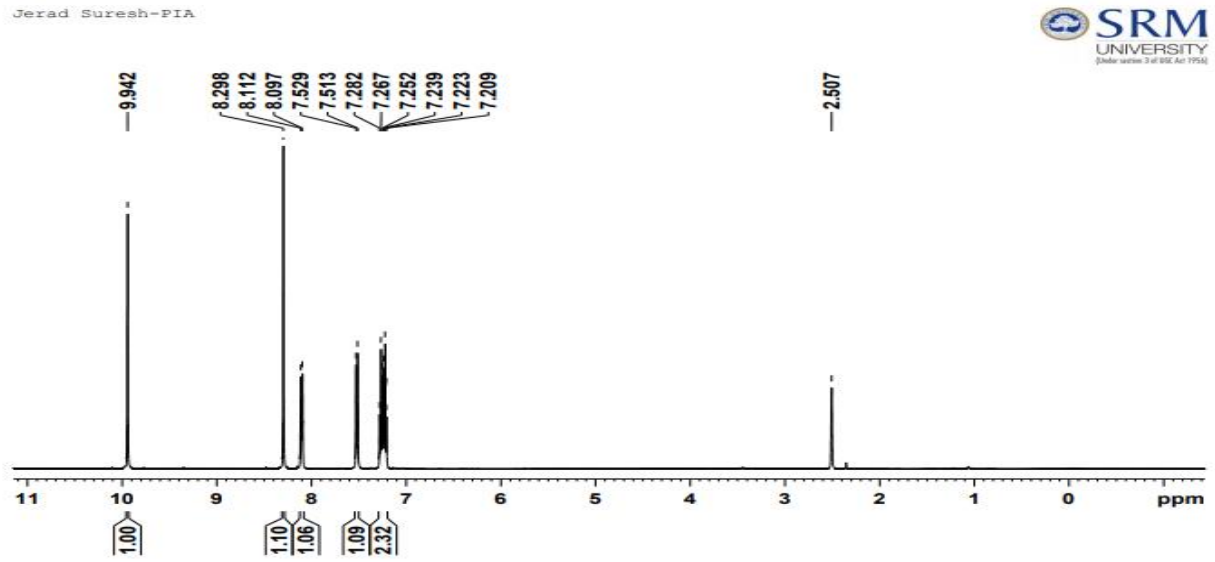


Table No :13 INTERPRETATION OF NMR:

S.No	δ VALUE (PPM)	NUMBER OF PROTONS
1	δ7.3	2 Protons
2	δ7.5	1 Protons
3	δ8.1-8.4	2 Protons
4.	δ9.8	1 Proton

BIOLOGICAL EVALUATION

The anti-tubercular activities of the synthesized compounds were determined by MicroplateAlamar Blue Assay method (MABA). The organism used in the study is Mycobacterium tuberculosis H37Rv. All the synthesized compounds showed anti-mycobacterial activity in varying degrees against the organism tested. The data pertaining to these observations are presented.

TABLE 14: ANTI-TB RESULTS

SL. NO	SAMPLES	100 µg/ml	50µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	DCP1	S	S	S	S	S	S	S	R
2.	DCP2	S	S	S	R	R	R	R	R
3.	DCP3	S	S	S	R	R	R	R	R
4.	PIA	S	S	S	S	R	R	R	R

NOTE:

S - Sensitive

R - Resistant

Strain used: ***M.tuberculosis***(H37 RV strain): ATCC No- 27294.

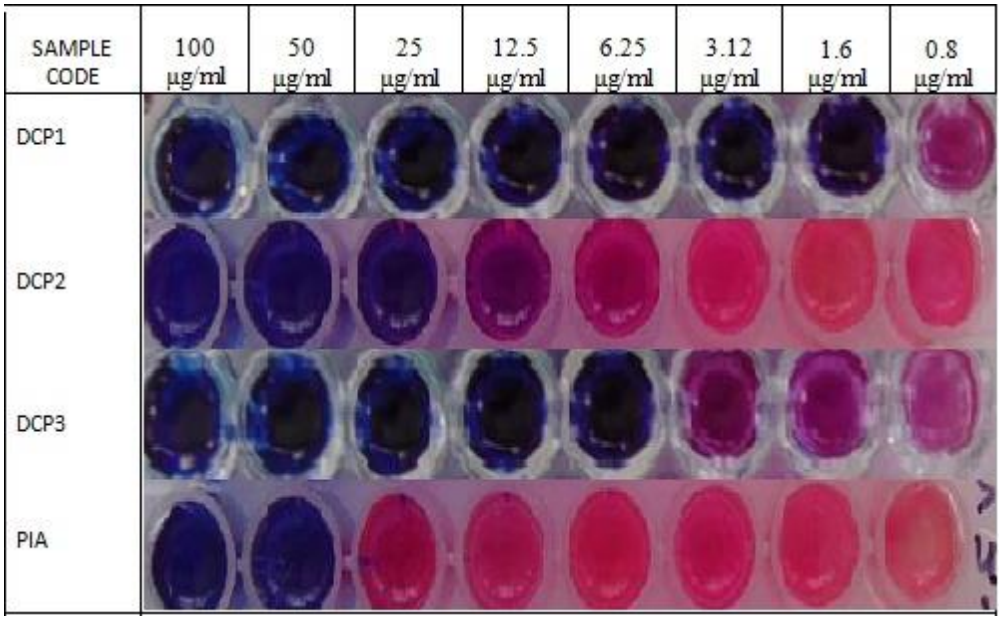
Here are the ***standard values*** for the Anti-Tb test which was performed.

Pyrazinamide- 3.125µg/ml

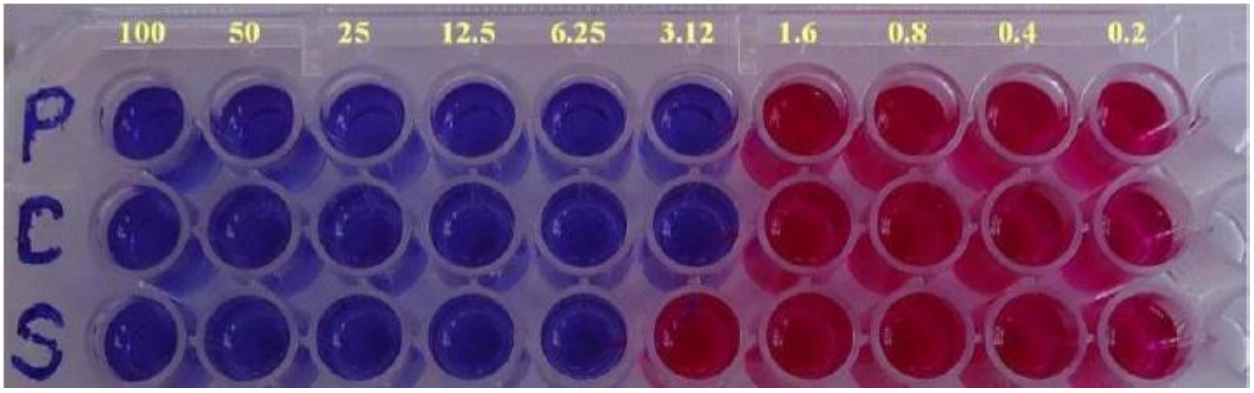
Streptomycin- 6.25µg/ml

Ciprofloxacin-3.125µg/ml

SNAP SHOT SAMPLE DRUGS :



SNAP SHOT OF STANDARD DRUG PHOTOGRAPH:



ACUTE ORAL TOXICITY STUDY:

Table15 . Acute oral toxicity study:

S.No	PARAMETERS	RESULTS
1	Toxic signs	Absent
2	Pre-terminal deaths	Nil
3	Body weight	No specific change
4	Motor activity	Normal
5	Tremors	Absent
6	Convulsion	Absent
7	Straub reaction	Absent
8	Righting reflex	Present
9	Lacrimation and salivation	Normal
10	Unusual vocalization	Absent
11	Sedation	Absent
12	Body temperature	Normal
13	Analgesia	Absent
14	Ptosis	Absent
15	Diarrhoea	Absent
16	Skin colour	Normal
17	Respiration	Normal
18	Scratching	Absent
19	Aggressiveness and restlessness	Absent

Animals were observed for behavioral signs of toxicity like motor activity, tremor etc., and no significant toxic signs were observed during 14 days. The results of the acute toxicological studies revealed that the administration of 2 molecules by oral route upto 2000mg/kg/b.w did not produce any mortality it was tolerated.

CYTOTOXICITY EVALUATION:

When campared the compound DCP3 with DCP1, DCP1 showed decreased IC50 values. The drug Rifampicin was used as standard which showed IC50 value as 113 mcg/ml.

The table presented below showed IC50 values of DCP1 and DCP3.

Table :16 CYTOTOXICITY:

Concentration (µ/ml)	**DCP1**	**DCP3**
500	52.15	53.59
250	43.68	47.38
125	34.30	41.26
64.5	34.83	27.67
31.25	12.86	16.11
IC50 FROM PRISM	**389.7**	**319.3**

.

DISCUSSION

- According to literature review pyridine was chosen as basic nucleus for antitubercular activity.
- Schiff bases, now a days screened for various pharmacological activities.
- The synthesized compounds labeled DCP1, DCP2, DCP3 and PIA have pyridine nucleus which imparts antitubercular activity.
- 3,5-dichloro 4-amino pyridine was taken as primary amine which allowed react with various substituted aldehydes , they form Schiff bases.
- The compound DCP2 showed good docking score which chemically called **(*E*)-1-(4-chlorophenyl)-*N*-(3,5-dichloropyridin-4 yl)methanimine**
- If the binding is less, the compound having high binding affinity towards target protein.
- InhA is enzyme that belong to Fatty Acid Synthase , that found to be one of critical enzyme essential for the synthesis of mycolic acid ,which is prerequisite for the formation of Mycobacterial cell wall.

SUMMARY

- Inh A(enoyl-ACP reductase) (2h9i) a critical enzyme for the growth of Mycobacterium tuberculosis was chosen for our study after review of literature.
- Candidate molecules were designed and docked against 2h9i protein using AUTO DOCK ®1.5.6 software.
- 4 Molecules with good Docking score (lower binding energy) and interactions were shortlisted for synthesis. Reaction conditions were optimized.
- The selected molecules were subjected to toxicity prediction assessment by OSIRIS ®software.
- Compounds were synthesized by conventional method and labeled as DCP1, DCP2, DCP3, PIA. Purity of the synthesized compounds was ensured by repeated recrystallisation and purification by column chromatography. Further the compounds were evaluated by TLC and Melting point determination.
- The characterization of the synthesized compounds was done using Infra-red, Nuclear Magnetic Resonance (H1 NMR, C13 NMR) and Mass spectroscopic methods (LC-MS,).
- The pure compounds were screened for *In- Vitro* Anti- mycobacterial activity by MicroplateAlamar Blue Assay (MABA). All compounds showed significant anti-mycobacterium activity.
- The synthesized compound DCP1 were active at 1.6µg/ml, which were comparable into the known anti-TB drugs: Pyrazinamide - 3.125µg/ml, Ciprofloxacin - 3.125µg/ml and Streptomycin - 6.25µg/ml. The compounds DCP2 and DCP3 were active at 12.5µg/ml.
- The IC50 values synthesized compounds DCP1 and DCP3 were 389.7 µg/ml and 319.3 µg/ml respectively

CONCLUSION

- Our work concludes that our synthesized molecules are effective in inhibiting enzymeInhA (enoyl-ACP reductase) (2h9i) which is important for the growth of Mycobacterium tuberculosis.
- All the 4 compounds gave Docking score between -5 to -8 kcal/mol. This goes to prove that 2h9i is a critical enzyme for anti-mycobacterial lactivity.
- The minimum inhibitory concentration of the 4 synthesized compounds against H37RV ranged from 50 to 1.6 μg/ml.
- Further structural refinement to the structure of the synthesized compounds is expected to yield promising molecules against the pathogen Mycobacterium tuberculosis.

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Proceedings of the Chairperson, Institutional Animal Ethics Committee, Madras Medical College, Chennai – 3.

Present: Dr.Sudha Seshayyan, M.B.B.S, M.S (Anatomy)

Roc. No. 19/ AEL/IAEC/MMC Date: 15.03.2018.

Sub: Animal Experimental laboratory – IAEC – research Project – approval – regarding.

Ref: IAEC meeting held on 06.09.2017.

The following order is based on the meeting held on 06.09.2017 and the addendum issued on 15.03.2018.

Project ID.	1 9/17.
CPCSEA registration number	1917 / ReBi/S/16/CPCSEA /25.10.2016
Name of the Researcher	S.SURESH KUMAR M. Pharm II year, Department of Pharmaceutical Chemistry
Name of the Guide	Dr.A.Jerad Suresh,M.Pharm, Ph.D.,
Title of the project	Design, Synthesis, Characterization and Biological Evaluation of some Novel Pyridine Derivatives as Anti-Tubercular Agents against InhA
Date of submission of proposal to IAEC	01.08.2017
Date on which IAEC conducted	06.09.2017
Date of submission of modified proposal (if applicable)	14.03.2018
Date on which approved	15.03.2018
Validity of the approved proposal	1 year
Remarks	Albino mice – Female 12 numbers approved.

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai -3

To,
Dr.A.Jerad Suresh,M.Pharm,Ph.D.,
Prof. & Head of Dept of Pharmaceutical Chemistry,
College of Pharmacy,
MMC, Chennai -3.

Copy to
Special Veterinary Officer, Animal Experimental Laboratory
Madras Medical College, Chennai – 3.

"Skill and Will to Make and Serve Quality Pill"

69th IPC 2017

CHANDIGARH

22nd - 24th December, 2017

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This is to certify that

Prof./Dr./Mr./Ms. S. SURESH KUMAR

has participated as Delegate / Volunteer

in the 69th Indian Pharmaceutical Congress

held at Chitkara University, Rajpura from December 22nd to 24th, 2017.

Dr. Mahesh Burande	Dr. Shailendra Saraf	Dr. Dhirender Kaushik	Dr. Ashish Baldi
President - IPCA	Chairman - LOC	Organizing Secretary	Chairman, Registration Committee - LOC

Organised by : Indian Pharmaceutical Congress Association (IPCA)

Hosted by : Association of Pharmaceutical Teachers of India (APTI)

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Theme Quality Pharmaceuticals and Patient Welfare

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Andhra University, Visakhapatnam, A.P. during 16th – 18th December 2016.

Mr. S.V. Veerramani	Dr. Rao Vadlamudi	Dr. T.V. Narayana	Dr. G. Nagarjuna Reddy
President, IPCA-2016	LOC, Chairman	LOC. Secretary	Chairman, Reg. Com.

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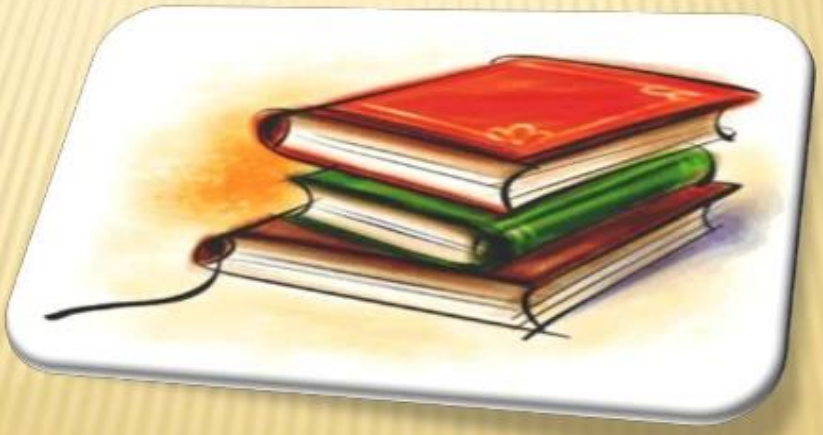
Introduction

AIM AND PLAN OF WORK

LITERATURE REVIEW




MATERIALS &
METHODS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Summary & Conclusion


REFERENCE

Annexure